J Endocrinol Reprod 11 (2007) 2: 86 - 99 **Original Article**

Prepuberal - onset hypothyroidism induces reversible boost in lipogenic activity in Sertoli cells of adult rats

R.C.R. Udayakumar, F.F. Antony, J. Anbalagan, R. Neelamohan, A. J. Stanley and M.M. Aruldhas [@]

Department of Endocrinology, Dr. ALM Post-Graduate Institute of Basic Medical Sciences, University of Madras Taramani Campus, Chennai - 600 113, India.

Summary

Sertoli cells, the major site of intermediary metabolism in the testis, are the target for thyroid hormone action, though thyroid hormone receptors are present in Leydig cells also. We have earlier shown specific changes in basal and LH-induced steroidogenic activity in Leydig cells of adult rats with transient prepuberal - onset hypothyroidism. In this paper we report the response of Sertoli cell lipids and lipogenic enzymes in adult rats to prepuberal onset hypothyroidism. Hypothyroidism was induced in 30 day old rats by surgical thyroidectomy. After 60 days, one group of hypothyroid rats was allowed to attain euthyroidism by L-thyroxine (T_4) replacement therapy (intramuscular injection of 6µg T_4/100 g body weight for thirty days) and sacrificed. Sertoli cells were isolated by enzymatic dispersion and the concentration of various lipid fractions and the activities of lipogenic enzymes were assessed. Hypothyroidism increased Sertoli cell total lipids and total cholesterol, glycereides and phospholipids. Of the lipogenic enzymes, activities of glucose-6-phosphate dehydrogenase, 6-phospho gluconate dehydrogenase and malic enzyme decreased, whereas those of $NADP^+$ isocitrate dehydrogenase, ATP-citrate lyase, fatty acid synthase and α - glycerophosphate dehydrogenase increased. T_4 replacement to thyroidectomized rats maintained normal levels of most of the parameters. The present study indicates that thyroid hormones have an inhibitory effect on Sertoli cell lipogenesis. It is suggested that accumulation of lipids in Sertoli cells may be one of the biochemical mechanisms underlying prepuberal–onset hypothyroidism–induced male infertility, which can be corrected with timely treatment of T_4 to maintain euthyroidism.

Key words: Hypothyroidism; lipids; lipogenic enzymes; Sertoli cells; testis; thyroxine.

Introduction

Mammalian testis is now considered as a target organ for thyroid hormones (Jannini et al., 1995; Longcope, 2000; Aruldhas, 2002). However, till 1980 thyroidologists, reproductive biologists and endocrinologists attributed the effect of thyroid hormones, if any, on mammalian testicular function to its indirect effect on general body metabolism (Myant, 1964) since thyroid hormones failed to increase oxygen production from the testis *in vitro* (Barker and Klitgaard, 1952), and attempts to identify specific thyroid hormone receptors in testis were not successful (Oppenheimer et al., 1974). However, a series of studies, which emanated from our laboratory on testicular intermediary metabolism in hypo- and hyperthyroid rats, suggested direct action of thyroid hormones on testis (Aruldhas et al., 1982a, b, 1983, 1984, 1986a, b). This idea gained support from the findings of Palmero et al. (1988, 1992, 1993, 1995) that Sertoli cells of immature rats and piglets have specific T_3 receptors (TR), and are under the homologous regulatory control of thyroid hormones, thus qualifying these sustentacular cells as the testicular target of T_3. These authors also showed that thyroid hormones play an essential role in regulating the development and functions of Sertoli cells (Palmero et al., 1989). Now, it has become an established concept that thyroid hormone(s) is one of the important regulators of testicular growth, maturation and function, along with gonadotrophins and sex steroids (Jannini et al., 1995; Huhtaniemi, 1999; Laslett et al., 2000; Longcope, 2000; Manna et al., 2001; Aruldhas, 2002; Mendis-Handagama and Ariyaratne, 2005; Jansen et al., 2007).

The highest expression of both TRα1and TRβ1 in rat Sertoli cells has been demonstrated during early neonatal life when thyroid hormone secretion is high (Palmero et al., 1989; Jannini et al., 1990, 1994; 1999, 2000; Bunick et al., 1994; Buzzard et al., 2000; Canale et al., 2001; McCoard et al., 2003). Though most of the studies on expression of TR in rat Sertoli cells suggest diminution of the same by the third week of postnatal life, coinciding with the cessation of Sertoli cell proliferation, and become undetectable in adults (Jannini et al., 1990, 1994, 1995, 1999, 2000; Cooke, 1995; Buzzard et al., 2000; Canale et al., 2001), immuno-histochemical studies

Correspondence to be addressed to: Dr. M. Michael Aruldhas (E-mail: aruldhas@unom.ac.in)

have shown the presence of TR in the interstitium of adult rat testis also (Tagami et al., 1990; Macchia et al., 1990). Though TRα2, a variant of TR β, is not expressed in Sertoli cells of normal adult rats, transient neonatal hypothyroidism induces its expression (Jannini et al., 1999). TRα2 was also isolated from adult human testis (Benbrook and Pfahl, 1987). TRα2 does not bind T_3, whereas it has a negative effect on other TR isoforms (D'Armiento and Jannini, 1992; Jannini et al., 2000). Studies from our laboratory and a few others on steroidogenesis and intermediary metabolism indicate that adult rat testis is also vulnerable to changes in thyroid hormone status (Aruldhas et al., 1982a, b, 1983, 1984, 1986a, b; Ando et al., 1990; Biswas et al., 1994; Antony et al., 1995), and thyroid hormones modulate Leydig cell function, and androgen and estrogen receptors in Sertoli cells of neonatal rats (Antony et al., 1995; Jana et al., 1996; Panno et al., 1996a, b).

Testis being a specialized organ, regulation of its metabolic activities might undergo modification to suit its specific needs. Sertoli cells, which play a pivotal role in regulating spermatogenic and steroidogenic functions (Skinner, 1991; Bardin et al., 1994; Griswold, 1998), are the major site of intermediary metabolism in the testis. The Sertoli cells provide nutrients for the dividing germ cells, which are in close association during the progression of spermatogenesis (Steinberger and Steinberger, 1971; Fawcett, 1975; de Kretser and Kerr, 1994). Since Sertoli cells nurse the spermatogonia, this relationship can in turn influence the development of spermatids (Skinner and Griswold, 1980; Skinner, 1991, 2005). Apart from nutrients, the paracrine growth factors from Sertoli cells such as insulin-like growth factor (IGF) system components, epidermal growth factor (EGF) and transforming growth factor (TGF) also regulate germ cells (Sharpe, 1993). While immature Sertoli cells secrete estradiol (E_2) and anti-Müllerian hormone(AMH), mature Sertoli cells secrete 5α - dihydrotestosterone (DHT) (de Kretser and Kerr, 1994), the enzyme γ–glutamyl transpeptidase and proteins such as androgen binding protein (ABP) and transferrin, which are under the regulation of FSH as well as testosterone. Sertoli cells also secrete the peptide hormones inhibin and activin (Sharpe, 1993; Sharpe et al., 2005).

Early studies from our laboratory have established that thyroid hormones have specific effect on different testicular lipids and lipogenic enzymes in prepuberal, puberal and adult rats (Aruldhas et al., 1983, 1984, 1986a,b). In another study we reported specific changes in basal and LH-induced steroidogenic activity in Leydig cells of adult rats subjected to prepuberal-onset hypothyroidism (Antony et al., 1995). In this paper, we report inhibitory effect of thyroid hormones on Sertoli cell lipids and lipogenic enzymes *in vivo* in mature rats subjected to hypothyroidism from prepuberal age onwards.

Materials and Methods

Animals

Thirty days old Wistar rats from our animal stock were used for the present study. Rats maintained in a well-ventilated animal room with 12 ± 1 hr dark and light schedule were provided with pellet food (Hindustan Lever Limited, India) and clean drinking water *ad libitum*. They were divided into the following groups (each group, n=15).

Group I : Euthyroid control rats

- 60days old
- 90 days old

Group II : Hypothyroid rats

- 60 days old
- 90 days old

Group III : Hypothyroid rats reverted to euthyroid state

Induction of hypothyroidism

The experiments were approved by the Institutional Animal Ethics Committee. Hypothyroidism was induced by surgical thryoidectomy under deep anesthesia. Thyroidectomised rats were supplemented with 1% $CaCl_2$ in drinking water to compensate the loss due to the removal of parathyroid glands along with thyroid gland. Hypothyroid rats were reverted to euthyroid state by supplementing T_4, at a daily dose of 6 µg/100g body weight (through intramuscular route) from day 31 post-thyroidectomy for 30 days (Aruldhas et al., 1982a; Antony et al., 1995).

Control rats for thyroidectomised group were sham-operated and those for T_4 treatment group were treated with the vehicle (alkaline saline, pH 7.8, daily) during the experimental period. The total duration of experiment was 60 days. At the end of the experiments, the rats were anaesthetized and blood was collected, serum separated and frozen until analysis. The rats were, then, dissected to remove the testes.

Isolation of Sertoli cell-enriched preparation

Testes were decapsulated with fine sterile forceps and Leydig cells were removed using collagenase under an environment of 95% O_2 and 5% CO_2 as described elsewhere (Antony et al., 1995), and thereafter the Sertoli cells were separated (Steinberger et al., 1975). Briefly, seminiferous tubules were resuspended in Hank's balanced salt medium containing trypsin (0.5 mg/ml) and DNAse (1mg/ml) and incubated for 10 min at 37°C. The suspension was shaken gently for 5 min, homogenized using a Teflon homogenizer and filtered through a 75µm metal grid to remove germ cell contaminants. The Sertoli cell-enriched preparation present in the mesh had a purity of 80-85%. The main contaminants were different types of germ cells.

Extraction of intratesticular testosterone

Intratesticular testosterone was extracted following the method of Jean-Faucher et al. (1985). The decapsulated testis was homogenized in N-ethylmalemide (5mM), which inhibits the transformation of testosterone into DHT, and testosterone was extracted in ethyl acetate: iso-octane glycol (2:1, w/v). The second extract was allowed to percolate into the column under nitrogen at low pressure. Progesterone in the extract was eluted with 3.5 ml iso-octane under nitrogen at low pressure and discarded. After this, testosterone was eluted with 3.5 ml benzene (40%) in iso-octane, dried under nitrogen and dissolved in 2 ml gelatine phosphate buffer (pH 7.0). Recovery of testosterone in this procedure was upto 80%.

Hormone assays

All hormones were assayed by radioimmunoassay (RIA) as explained earlier (Antony et al., 1995). Testosterone was assayed by liquid phase RIA using protocol and reagents (Batch No. K07981) received from World Health Organization (WHO). The maximum binding of the testosterone antiserum was 40% and its cross reactivity to cortisol, dihydrotestosterone, androstenedione and androstenediol was 0.001%, 14%, 0.8% and 6%, respectively. The inter-assay and intra-assay variations were 6% and 4%, respectively. The sensitivity of the assay was 0.3 pg /ml.

Serum TSH and FSH were assayed by liquid phase RIA using a double antibody technique as per the procedure of WHO (Sufi et al., 1980) using specific rat antigens (NIDDK- rTSH – I – 9 – AFP 730 8C; NIDDK – (rFSH-I.8-AFP-114543), antibodies NIDDK-anti rTSH-S-S-C21381; NIDDK-anti rFSH – S-H- AF P-C 097881) and reference (NIDDK- rFSH-RP2-AFP-5153 B; NIDDK-rFSH-RP2-AFP-4621B). TSH concentration is expressed as ng/ml of NIDDK- r TSH-RP2 standard and FSH as ng/ml of NIDDK- rTSH-RP2 standard. The cross reactivity of TSH antibody to other hormones was 5.6% for rLH, 1.3% for rFSH, <0.003% for rPRL and 0.7% rGH. The cross reactivity of FSH antibody to other hormones was <0.5% for rLH, <0.2% for rTSH, 0.01% for rPRL and rGH. The intra-assay variations were 4.7-6.9% for rTSH and 3.7-8.9% for rFSH; inter-assay variations were 8.2% for TSH and 12.2% for rFSH. Sensitivity of assays was 0.01ng/ml and 0.2 ng/mL for TSH and FSH, respectively.

Total and free T_4 and T_3 were assayed by solid phase RIA using commercial kits obtained from Diagnostics Products Corporation, Los Angeles, USA. Sensitivities of these assays were: 0.25µ g/dL for total T_4, 0.01 ng/dL for free T_4, 7 ng/dL for total T_3 and 0.12 pg/mL for free T_3.

cAMP was assayed by liquid phase RIA using the kit obtained from Amersham Intl., Buckinghamshire, UK, following the method of Steiner et al. (1972) The intra - and interassay variations of the assay was 4.0 - 5.5% and < 11%, respectively. The sensitivity of the assay was 0.05 pmol / tube.

Enzyme assays

The activities of glucose-6-phosphate dehydrogenase (D-glucose 6-phosphate NADP-1-oxidoreductase, E.C. 1.1.1.49) (Löhr and Waller, 1974), 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate NADP–2–oxido-reductase (E.C. 1.1.1.4) (King, 1974), NADP+-isocitrate dehydrogenase (D-Isocitrate-NADP+ oxidoreductase, E.C.1.1.1.42) (Bernt and Bergmeyer, 1974), malic enzyme (NADP+ malate dehydrogenase, E.C.1.1.1.40) (Ochoa, 1955) and ATP citrate lyase (ATP citrate oxaloacetate lyase, E.C. 4.1.38) (Srere, 1962) were determined spectrophotometrically. Glycerophosphate dehydrogenase (L-glycerol-3 phosphate: NAD oxidoreductase, E.C. 1.1.1.8) activity was assayed following the method described by White (1975), and fatty acid synthase (E.C. 2.3.1.38) adopting the method of Smith and Abraham (1975).

Determination of lipids

The total lipids were extracted in chloroform: methanol (2: 1 v/v) containing butylated hydroxyl toluene

(0.01%) as an antioxidant (Folch et al., 1957) and determined colorimetrically using phosphor-vanillin reagent (Frings et al., 1972). Total glyceride glycerol, total phospholipids and total cholesterol were also determined colorimetrically (Aruldhas et al., 1986a, b).

Statistics

Students'*t* test was employed to test the statistical significance of the data from the different groups (Zar, 1954).

Results

Sertoli cell enzymes

Hypothyroidism decreased the specific activities of G-6-PDH, 6-PGDH and malic enzyme in Sertoli cells, whereas T_4 supplementation to thyroidectomized rats maintained normal activity of these enzymes. Unlike enzymes of the HMP- shunt pathway, activities of Sertoli cell $NADP^+$ - ICDH, α-glycerophosphate dehydrogenase, ATP-citrate lyase and fatty acid synthase increased in hypothyroid rats. T_4 supplementation to hypothyroid rats reverted activities of most of the enzymes to normal except for G-6-PDH and $NADP^+$ - ICDH, though they showed a trend towards the level in age matched control rats (Figs.1,2).

Sertoil cell concentration of lipids

Hypothyroidism brought about accumulation of total lipids and total cholesterol, glyceride glycerol and phospholipids in Sertoli cells, which returned to normal with the reversal to euthyroid state (Fig.3).

Sertoli cell concentration of cAMP

Thyroidectomy resulted in decreased concentration of cAMP in Sertoli cells, whereas T_4 supplementation to thyroidectomized rats restored its concentration at par with age matched control rats (Fig.2).

Hormones

Hypothyroidism brought about a significant decrease in serum total and free T_4 and T_3, while serum TSH increased. T_4 supplementation to thyroidectomized rats restored these hormones concentration to normal levels, except serum testosterone. Concentrations to normal levels. Hypothyroidism brought about decrease of serum FSH significantly, whereas the reversal to euthyroid condition resulted in maintenance of normal serum FSH tire (Table 1).

Serum and testicular testosterone and serum estradiol levels decreased in hypothyroid rats. T_4 supplementation to thyroidectomized rats restored these hormone concentrations to normal levels, except serum testosterone (Table 1).

Discussion

The data on serum TSH, T_4 and T_3 ascertain the induction of hypothyroidism and restoration of euthyroid status in untreated and T_4 treated thyroidectomized animals, respectively. The data on Sertoli cell enzymes involved in NADPH generation suggest an enzyme-specific differential influence of thyroid hormones. The present *in vivo* study reveals that G-6-PDH, 6-PGDH and malic enzyme ($NADP^+$ - MDH) activities in Sertoli cells are under the stimulatory control of thyroid hormones, whereas $NADP^+$-ICDH alone is under an inhibitory control. This can be deduced from decrease in the activities of first three enzymes and increase in the activity of the latter observed in thyroidectomized rats, and restoration of all enzyme activities to the respective normal / near normal levels when the animals were reverted to euthyroid state (except G-6-PDH and $NADP^+$ - ICDH, which did not reach normal levels in response to T_4 supplementation). Though the reason for the differential response of $NADP^+$ - ICDH and the failure of it and G-6-PDH to revert to normal levels with reversal of euthyroidism is not clear at present, it indicates that there is enzyme specific effect of thyroid hormones on NADPH generating systems in Sertoli cells. Testicular testosterone, and serum FSH and estradiol, well known regulators of Sertoli cell, and the concentration of cAMP in Sertoli cell, reached normal levels with T_4 replacement. Probably, other regulators of Sertoli cell functions may act synergistically with thyroid hormones to maintain the enzyme activity, which might not have reached the optimum level. Nevertheless, the specific inhibitory effect of thyroid hormone on $NADP^+$- ICDH activity in Sertoli cells of rats is clearly attested by the increased activity observed in thyroidectomized rats and the decrease in the same in response to T_4 supplementation.

NADPH being an essential co-enzyme for lipogenesis associated with malonyl CO-A synthetic pathway and fatty acid chain elongation (Mayes, 1988), thyroidal influence on these enzymes may be expected to reflect on Sertoli cell lipid composition. Among all four enzymes involved in NADPH generation, $NADP^+$ – ICDH appears to be specifically associated with lipogenesis in Sertoli cells of rats. This may be inferred from the observed consistency between responses of Sertoli cell lipids and the specific activity of $NADP^+$ - ICDH under hypo- and

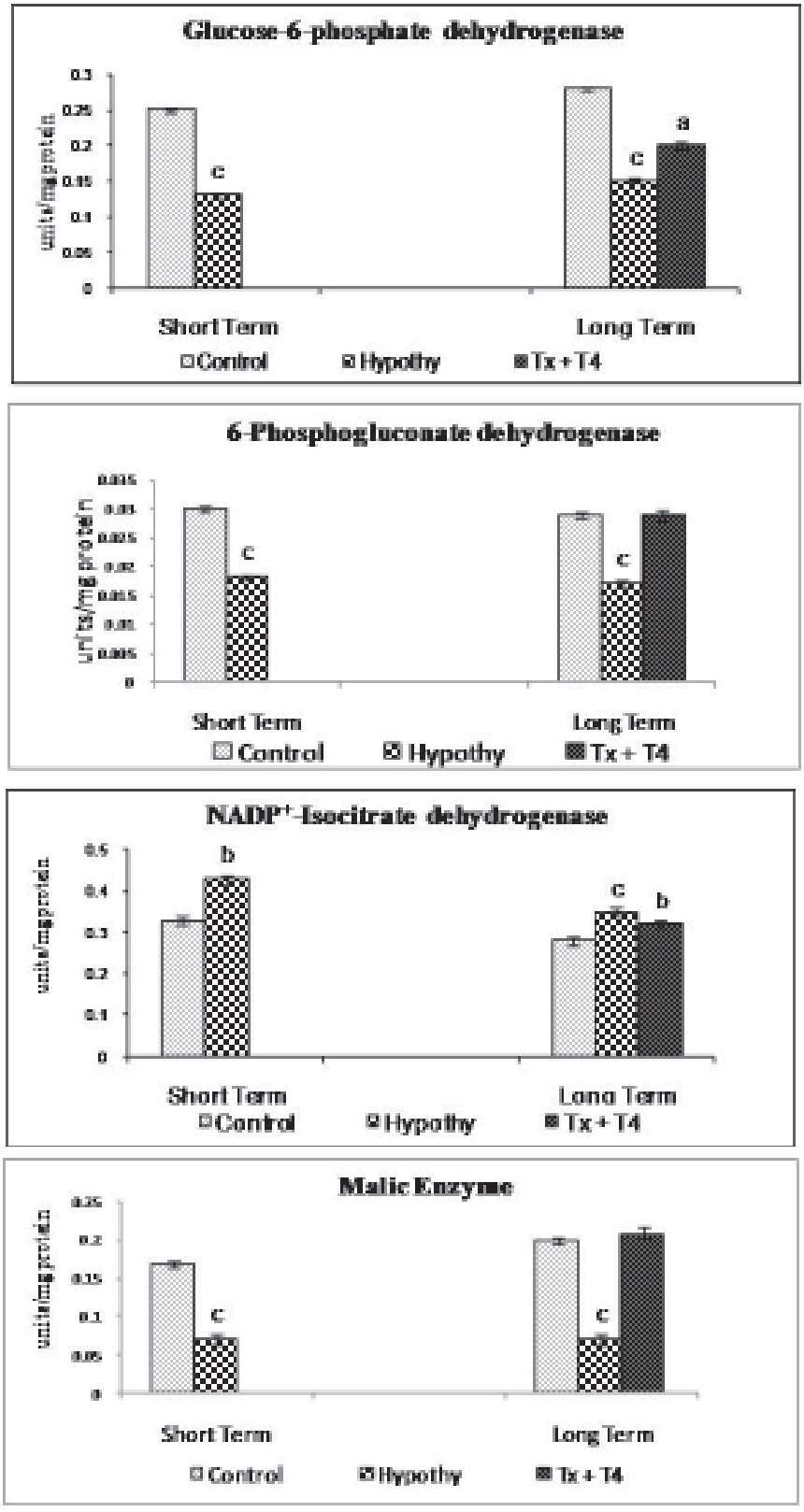

Fig.1: Effect of prepuberal-onset hypothyroidism and T_4 replacement on the specific activity of NADPH generating enzymes in Sertoli cells of adult rats. All experiments were started on 31dpp. Short term experiments was for 30 days and long-term for 60days. T_4 replacement was begun on 61dpp. Values are mean± SEM. n= 12. a,b,c-statistical significance at $p<0$ .050, 0.01 & 0.001 against the respective control.

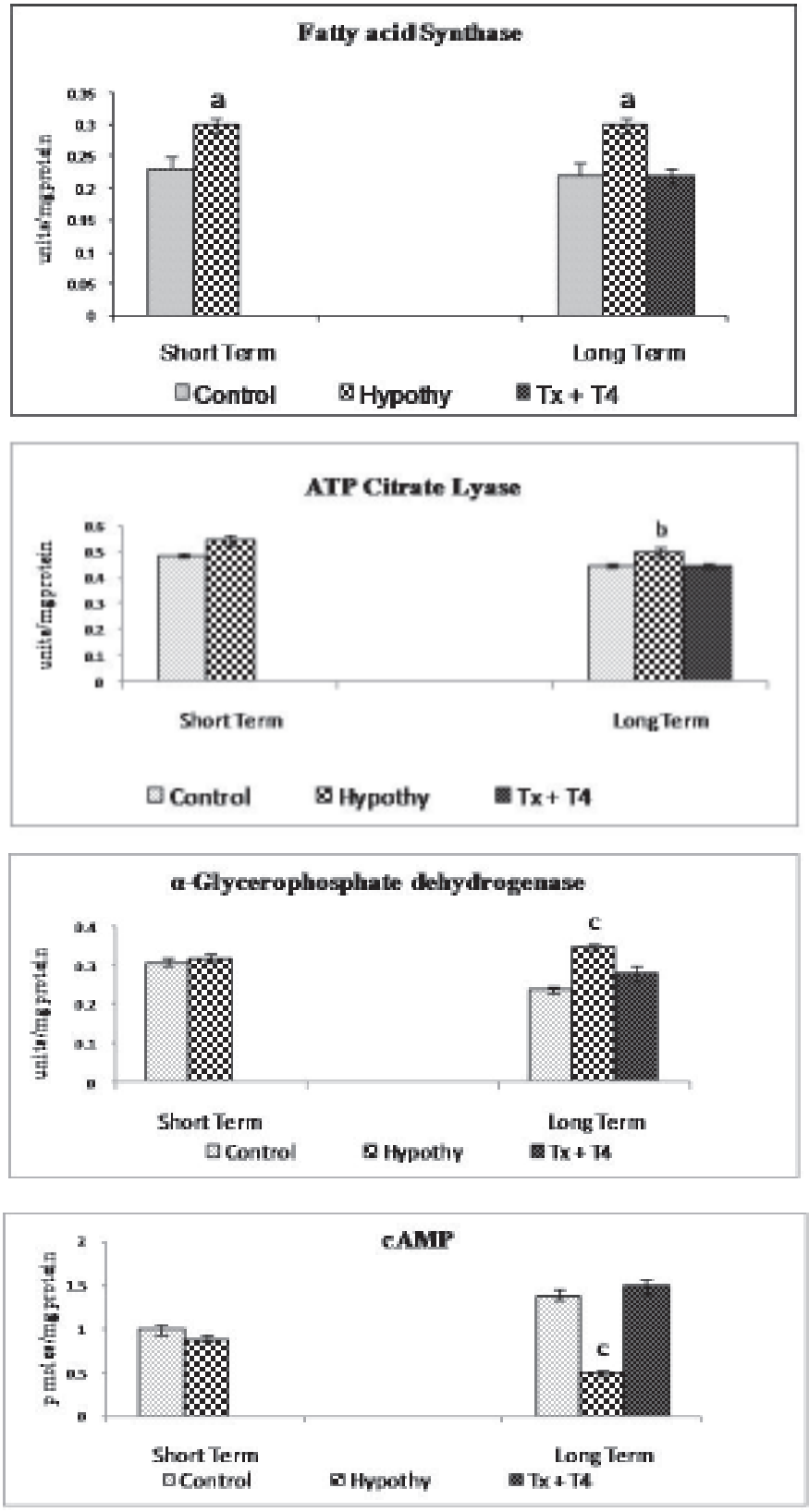

Fig. 2: Effect of prepuberal-onset hypothyroidism and T_4 replacement on the specific activity of lipogenic enzymes and cAMP concentration in Sertoli cells of adult rats. All experiments were started on 31dpp. Short term experiments was for 30 days and long-term for 60days. T_4 replacement was begun on 61dpp. Values are mean± SEM. n= 12.a, b, c - statistical significance at p<0 .05, 0.01 & 0.001 against the respective control.

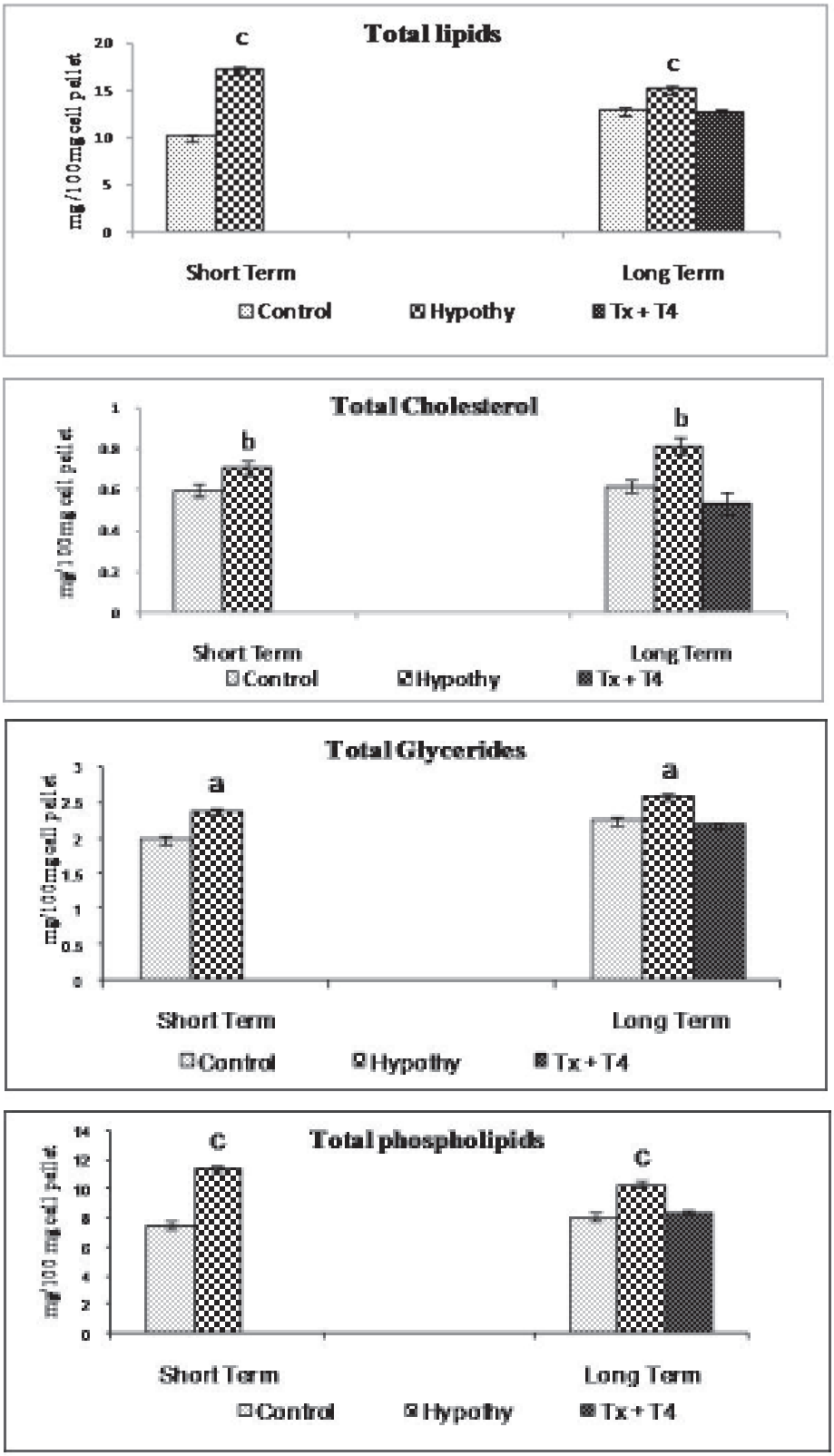

Fig 3: Effect of prepuberal-onset hypothyroidism and T_4 replacement on the concentration of lipids in Sertoli cells of adult rats. All experiments were started on 31dpp. Short term experiments was for 30 days and long-term for 60days. T_4 replacement was begun on 61dpp. Values are mean± SEM. n= 12. a, b, c-statistical significance at p<0 .05, 0.01 & 0.001 against the respective control.

Table 1: Serum and testicular hormones in control, thyroidectomized and T_4 - supplemented thyroidectomized rats. Values are mean ±SEM of 15 animals.

	Puberal rats		Adult rats		
	Control	Hypothyroid	Control	Hypothyroid	Tx + T_4
Total T_4 (µg/dL)	4.9±0.10	0.5±0.01[a]	6.5±0.80	0.7±0.10[a]	5.2±0.20
Total T_3 (mg/dL)	80.3±1.50	14.2±6.5[a]	87.2±6.80	12.1±3.30[a]	75.4±6.4
FT_4(ng/dL)	0.9±0.05	0.23±0.011[a]	1.2±0.08	0.12±0.001[a]	1.12±0.04
FT_3(pg/mL)	1.1±0.06	0.03±0.01[a]	1.4±0.10	0.21±0.012[a]	1.5±0.10
FSH(ng/mL)	100.6±7.2	12.4 ±0.94[a]	114.8±7.2	42.5±2.12[a]	108.2±4.2
TSH(ng/mL)	7.5±1.05	87±12.5[a]	10.9±1.10	60.7±6.30[a]	10.8±1.10
Serum T (ng/mL)	2.3±0.14	1.6±0.09[a]	3.2±0.18	0.5±0.03[a]	1.7±0.009[a]
Testicular T (ng/100mg tissue)	70.7± 4.0	57 ±4.3[a]	65.2 ± 2.1	31.3 ± 4.7[a]	61.4 ± 3.6
Serum E_2 (ng / mL)	4.9± 0.4	2.5 ±0.2[a]	6.5 ± 0.60	3.2 ± 0.43[a]	6.2 ± 0.70

Rats were thyroidectomized surgically on 30 dpp and sacrificed after 30 or 60 days. T_4 (6µg / 100 g body wt) was injected *i.m.* from day 31 after thyroidectomy for 30 days. For each parameter, means with superscripts are significantly different from controls. a = $p < 0.001$

euthyroid conditions, whereas an opposite trend was observed between Sertoli cell lipids and specific activities of G-6-PDH , 6-PGDH and malic enzyme. Specific activities of lipogenic enzymes like ATP–citrate lyase, α-glycerophosphate dehydrogenase and fatty acid synthase showed similar response as $NADP^+$ - ICDH. ATP–citrate lyase catalyzes the formation of acetyl Co-A, while fatty acid synthase catalyses the conversion of acetyl Co-A into fatty acids, whereas α-glycerophosphate dehydrogenase catalyses the conversion of dihydroxyacetone phosphate to glycerophosphate in the triacyl glycerol synthesis (Banks et al., 1977; Deshpande et al., 1985; Mayes, 1988). Therefore, taking together from the stimulation of these lipogenic enzymes along with $NADP^+$- ICDH activity observed in the present study, it is suggested that prepuberal - onset hypothyroidism augments lipogenesis in Sertoli cells of puberal and adult rats. This proposal gets entrenched by the accumulation of different classes of lipids observed in Sertoli cells of these rats. Hypothyroidism–induced augmentation of lipogenesis and accumulation of lipids in Sertoli cells of thyroidectomized rats are reversible since T_4 treatment to thyroidectomized rats decreased the activities of lipogenic enzymes and maintained them at normal levels. Thus, accumulation and diminution of lipids in hypothyroid and T_4 treated hypothyroid rats, respectively, can be attributed to parallel changes in lipogenesis. The failure of G-6-PDH and $NADP^+$- ICDH activity to revert to complete normal status suggests that hypothyroidism-induced changes in Sertoli cell lipids need not be reflected uniformly in all enzymes of the lipogenic pathway. The inhibitory effect of thyroid hormone on Sertoli cell ATP–citrate lyase observed in this study is consistent with the earlier studies on whole testis of immature and mature rats (Aruldhas et al., 1983, 1984).

Sertoli cell provides specific nutrients, including different lipid classes, to developing germ cells in a stage-specific manner (Means et al., 1978; Jutte et al., 1982; Rich and de Kretser, 1983; Ueno and Mori, 1990; Skinner, 1991; Bardin et al., 1994; de Kretser and Kerr, 1994; Griswold, 1998). Therefore, changes in utilization of Sertoli cell lipids by germ cells due to disruption of spermatogenic activity and increased germ cell degeneration in hypothyroid rats (Amin and EI-Sheikh, 1977; Aruldhas, 1981; Chowdhury and Arora, 1984; Francavilla et al., 1991; Simorangkir et al., 1997) may also lead to accumulation of lipids in Sertoli cells. Similarly, the stimulatory effect of thyroid hormones on spermatogenesis (Aruldhas, 1981; Francavilla et al.,

1991) might have resulted in enhanced utilization of Sertoli cell lipids in T_4 replaced hypothyroid rats.

α-glycerophosphate dehydrogenase is considered as a marker for thyroid hormone action on liver and other peripheral organs (Nunez, 1988) . Oppenheimer et al. (1978) classified testis as a non-responsive organ to thyroid hormones since T_3 administration to mature rats failed to evoke any change in the activity of testicular α-glycerophosphate dehydrogenase. The data from the present study provides clear evidence for an inhibitory control of thyroid hormones on Sertoli cell α-glycerophosphate dehydrogenase. This is interesting since α-glycerophosphate dehydrogenase and ATP-citrate lyase were shown to be under a stimulatory control of thyroid hormones in non-reproductive organs as in the case of G-6-PDH and 6-PGDH (Oppenheimer et al., 1987). T_4 was shown to stimulate adipose tissue ATP-citrate lyase in hypophysectomized rats (Brown et al., 1966) and T_3 stimulated fatty acid synthase activity in rat liver (Nunez, 1988). All these reports are inconsistent with the present study on Sertoli cells. Therefore, the discrepancy between the present study on Sertoli cells and the earlier studies on non-reproductive organs may be attributed to an organ-specific modified effect of thyroid hormones to suit the specific needs of the organ/cell types. Testis being a reproductive tissue, the effect of thyroid hormones on it may be different from the other non-reproductive target organs. Rao et al. (1984) reported that α-glycerophosphate dehydrogenase is non-responsive to T_3 in the breast tissue, attesting the idea of organ-specific effect of thyroid hormones on lipogenic enzymes.

Early studies from our laboratory on whole testis of rats subjected to similar experimental protocol (Aruldhas et al., 1983, 1984) showed decreased activities of G-6-PDH, G-6-PGDH and $NADP^+$-iso-citrate dehydrogenase in hypothyroid rats and increase in response to T_4 supplementation to thyroidectomized rats or in thyroid-intact rats made hyperthyroid, whereas LH and FSH levels decreased in both conditions. On the other hand, NAD/NADP-malate dehydrogenases and ATP-citrate lyase recorded increased and decreased activities in hypo- and hyperthyroid rats, respectively (Aruldhas et al., 1983, 1984). Based on these findings, we proposed a testis-specific inhibitory effect of thyroid hormones on ATP-citrate lyase and NAD/NADP-malate dehydrogenase, which are known to be under the stimulatory control of thyroid hormones in other organs. Interestingly, the present study on isolated Sertoli cells is consistent with our earlier studies, except for the inhibitory effect of thyroid hormones on the specific activity of $NADP^+$- ICDH isocitrate dehydrogenase in Sertoli cells. We have also observed an inhibitory effect of thyroid hormones on HMP-shunt enzymes and a stimulatory effect on $NADP^+$-iso-citrate dehydrogenases in Leydig cells (unpublished data). Taken together, all these suggest that thyroid hormones have cell-specific effect on testicular lipogenic enzymes, apart from their organ-specific effects. In general, the responses of lipogenic enzymes in Sertoli cells reflect their status in the whole testis.

The testis-specific effect of iodothyronines may be attributed to the specific type of TR present in it. TRα is the major thyroid hormone receptor in the testis, whereas TRβ is predominant in most of the non-reproductive organs (Nunez, 1988; Jannini et al., 1995). Transient neonatal hypothyroidism –induced boost in Sertoli cell population was shown to occur normally in TRβ-KO mice, whereas it failed in $TR\alpha_1$-KO mice, suggesting that T_3 regulates Sertoli cell proliferation through $TR\alpha_1$ rather than $TR\beta_1$ (Holsberger et al., 2005). Therefore, the differential responses of lipogenic enzymes between testis and other peripheral organs may be the result of difference in the isoforms of TR present in them.

Though FSH and testosterone are classical hormonal regulators of Sertoli cell functions (Fawcett, 1975; Means et al., 1978; Rich and de Kretser, 1983), the importance of thyroid hormones in Sertoli cell proliferation, maturation and function has come to stay (Cooke, 1995; Maran et al., 1999a, b). Sertoli cell lipids are known to have a definite correlation to FSH (Swerdloff et al., 1971; de Kretser et al., 1974). The data on FSH in hypothyroid rats do not tally with the observed changes in Sertoli cell lipids or lipogenic enzymes, except that of HMP shunt and malic enzymes. While serum FSH remained low in hypo- and hyperthyroid rats, lipid profiles and activities of lipogenic enzymes involved in fatty acid/acetyl Co-A synthesis increased in hypothyroid rats. A recent study from our laboratory on adult rats subjected to transient neonatal hypothyroidism for four weeks from birth showed increased FSH binding to its receptors in Sertoli cells (Venkatesh, 2004). This suggests that changes in circulating FSH due to altered thyroid hormone status may be overcome by Sertoli cells at the level of FSHR. Probably, there were specific changes in FSH signaling in rats of the present study, leading to the inconsistency observed between serum FSH and Sertoli cell lipids / lipogenic enzymes. The concentration of Sertoli cell cAMP, the classical second

messenger of FSH action, showed opposite trend to lipid profiles, i.e., its concentration decreased in hypothyroid rats and increased in T_4-treated thyroidectomized rats. Since cAMP is known to be lipolytic (Bierman and Glomset 1981; Hosh, 1988), the accumulation of lipid may also be attributed to a probable decrease in cAMP-mediated lipolysis in Sertoli cells of hypothyroid rats.

A positive association among serum thyroid hormones and FSH titers, serum and intra-testicular testosterone, Sertoli cell concentration of cAMP and specific activities of 6-PGDH, G-6-PGDH and malic enzyme could be seen in the present study. Probably, there is a synergistic action among thyroid hormones, testosterone and FSH in regulating these enzymes in Sertoli cells. On the contrary, a negative association could be observed among these hormones in relation to responses of Sertoli cell $NADP^+$- ICDH and enzymes involved in acetyl Co-A and fatty acid synthesis and lipids This inconsistency shall indicate that all the effects of thyroid hormones on Sertoli cells may not be mediated by FSH and testosterone.

Recent studies have shown that T_3 has a stimulatory effect on androgen receptor number and an inhibitory effect on estradiol receptor number in peripuberal rat Sertoli cells (Panno et al., 1996a, b). A probable decrease in Sertoli cell androgen receptors in hypothyroid rats may, therefore, be attributed to the specific changes observed in enzymes like G-6-PDH and G-6-PGDH in hypothyroid conditions.

T_3 is known to stimulate IGF-I production by cultured rat Sertoli cells from hypothyroid rats, which produce low level of IGF-I (Palmero et al., 1990). These authors have suggested that IGF-I mediates thyroid hormone-induced changes in testicular function. However, IGF is known to act like insulin and growth hormone (Palmero et al., 1990). Presumably, hypothyroidism-induced changes in Sertoli cell lipids and lipogenic enzymes are also mediated by changes in IGF-I or other paracrine factors.

Though the concentration of TRs in Sertoli cell becomes undetectable at puberty, expression of $TR\alpha_2$ persists till puberty but at a reduced rate (Jannini et al., 1995). Recently, it has been shown that the genomic locus of *c-erbAa*, the proto-oncogene encoding TRα and its variants, transcribes *rev-erbAa* mRNA in the prepuberal rats and is maximally expressed in adult rats (Jannini et al., 1995). The functional significance of expression of *rev-erbAa* mRNA in adult rats is not known at present. It is quite possible that the thyroid hormone-induced changes in adult rat testis are mediated by such receptors. A recent *in vitro* study conducted in our laboratory showed that T_3 modifies androgen binding protein (ABP) and lactate production by Sertoli cells isolated from puberal rats (Maran, 1997). The experimental rats of the present study were subjected to thyroid manipulation from prepuberal age onwards. Therefore, it is possible that altered thyroid status during functional maturation of the testis influences Sertoli cell function even at 90 days of age. Most of these changes were reversible since restoration of euthyroidism by 60 days of age maintained normal status in a majority of the parameters studied.

Sertoli cell lipids play an important role in germ cell survival as their plasma membrane lipids are derived from Sertoli cells (Ueno and Mori, 1990; Bardin et al., 1994). Sertoli cell lipids also serve as an alternative source of energy for the survival of germ cells (Jutte et al., 1982). Therefore, hypothyroidism-associated disruption in spermatogenic activity or infertility (Jannini et al., 1995; Longcope, 2000), may also be the result of altered Sertoli cell lipid metabolism.

In conclusion, this study reveals that thyroid hormones have an inhibitory effect on Sertoli cell lipogenesis, and prepuberal onset of hypothyroidism affects Sertoli cell lipid metabolism in mature rats. The effect of thyroid hormones on testicular lipids is specific since it differs from the effect seen in non-reproductive organs. Altered metabolism of Sertoli cell lipids may be one of the biochemical mechanisms underlying infertility in hypothyroid males. Importantly, the study reveals that hypothyroidism-induced changes in Sertoli cell lipids and lipogenic enzymes could be reversed by T_4 replacement therapy to maintain euthyroid state in hypothyroid rats. Therefore, appropriate iodothyronine therapy to hypothyroid subjects may help them to retain normal fertility.

Acknowledgments

This study was executed with financial support from University Grants Commission (UPE, SAP & ASIST programmes), Council of Scientific and Industrial Research (CSIR) and Department of Science and Technology (FIST scheme), New Delhi.

References

Amin SO and EI-Sheikh AS (1977) Pituitary-testicular function changes in hypo- and hyperthyroid male rats. *Acta Anat* 98:121-129.

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

Antony FF, Aruldhas MM, Udhayakumar RCR, Maran RRM and Govindarajulu P (1995) Inhibition of Leydig cell activity *in vivo* and *in vitro* in hypothyroid rats. *J Endocrinol* 144:293-300.

Aruldhas MM (1981) *Thyroid testicular interrelationship*. Ph.D. Thesis, University of Madras, Chennai, India.

Aruldhas MM (2002) Thyroid and the mammalian testis – an update. *Proc XX Symp Reprod Biol Comp Endocrinol*, Tiruchirappalli, India, Jan 7-9, pp15-18.

Aruldhas MM, Valivullah HM and Govindarajulu P (1982a) Specific effect of the thyroid on testicular enzymes involved in carbohydrate metabolism I. Hypothyroidism. *Int J Androl* 5:196-204.

Aruldhas MM, Valivullah HM and Govindarajulu P (1982b) Specific effect of thyroid hormone on testicular enzymes involved in carbohydrate metabolism. II. Hyperthyroidism. *Biochim Biophys Acta* 715:121-125.

Aruldhas MM, Valivullah HM and Govindarajulu P (1983) Effect of thyroidectomy on testicular enzymes of the pyruvate/malate cycle involved in lipogenesis. *Biochim Biophys Acta* 755:90-94.

Aruldhas MM, Vallivullah HM and Govindarajulu P (1984) Effect of thyroxine-induced hyperthyroidism on some testicular enzymes of the pyruvate/ malate cycle. *Biochim Biophys Acta* 797:143-146

Aruldhas MM, Balasubramanian K, Srinivasan N and Govindarajulu P (1986a) Role of thyroid on testicular lipids in prepubertal, pubertal and adult rats II: Hypothyroidism. *J Reprod Biol Comp Endocrinol* 6:41-51.

Aruldhas MM, Valivullah HM, Srinivasan N and Govindarajulu P (1986b) Role of thyroid on testicular lipids in prepubertal, pubertal and adult rats I: Hyperthyroidism. *Biochim Biophys Acta* 881:462-469.

Brown J, McLean P and Greenbaum AL (1966) Influence of thyroxine and luteinizing hormone on some enzymes concerned with lipogenesis in adipose tissue, testis and adrenal galnd. *Biochem J* 101:197-203.

Bunick D, Kirby J, Hess RA and Cooke PS (1994) Developmental expression of testis messenger ribonucleic acids in the rat following propylthiouracil-induced neonatal hypothyroidism. *Biol Reprod* 51:706–713.

Buzzard JJ, Morrison JR, O'Bryan MK, Song Q and Wreford NG (2000) Developmental expression of thyroid hormone receptors in the rat testis. *Biol Reprod* 62:664–669.

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

Chowdhury RA and Arora U (1984) Role of thyroid in testicular development of immature rat. *Arch Androl* 12:49-51.

Cooke PS (1995) Role of thyroid hormones in Sertoli cell mitogenesis and differentiation. *Mol Androl* 7:178-189.

D'Armiento M and Jannini EA **(**1992) Thyroid hormone action. In: Troncone L, Shapiro B, Satta MA and Monaco F (eds.), *Thyroid Disease: Basic Science, Pathology, Clinical and Laboratory Diagnoses*, pp145–154, CRC Press, Boca Raton, FL.

de Kretser DM and Kerr JB (1994) The cytology of the testis. In: Knobil E and Neill JD (eds), *The Physiology of Reproduction*, pp 1177-1290, Raven Press, NewYork.

de Kretser DM, Burger HG and Hudson B (1974) The relationship between germinal cells and serum FSH levels in males with infertility. *J Clin Endocrinol Metab* 38:787-793.

Deshpande N, Mitchell I, Maltini M, Boi A and Di Martino L (1985) The effects of the administration of tamoxifen, ethynyl estradiol and prednisolone on the activities of certain enzymes of carbohydrate metabolism in primary breast carcinoma *in vivo*. *Br J Cancer* 52: 241-244.

Fawcett DW (1975) The ultrastructure and functions of the Sertoli cell. In: Greep RO, Hamilton DW (eds), *Handbook of Physiology*, Section 7, Volume 5, *Endocrinology*, pp 21-55, American Physiological Society, Washington DC.

Folch J, Lees N and Solane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497-509.

Francavilla S, Cordeschi G, properzi G, Dicicco L, Jannini EA, Palmero S, Fugassa Endocrinology, Loras B and D'Armiento M (1991) Effect of thyroid hormone on the pre- and post-natal development of rat testis. *J Endocrinol* 129:35-42.

Frings CS, Fendley TW, Dunn RT and Queen CA (1972) Improved determination of total serum lipids by the sulpho-phospho-vanillin reaction. *Clin Chem* 18:673-674.

Griswold MD (1998) The central role of Sertoli cells in spermatogenesis. *Growth Hormone and IGF Research* 9:236-240.

Hoch FL (1988). Lipids and thyroid hormones. *Prog Lipid Res* 27:199-270.

Holsberger DR, Kiesewetter SE and Cooke PS (2005) Regulation of neonatal Sertoli cell development by thyroid hormone receptor alpha1. *Biol Reprod* 73:396–403.

Huhtaniemi IT (1999) Endocrine regulation of male reproduction. In: Wang (ed.), *Male Reproductive Function*, pp 1-8, Kluwer Academic Publishers, .

Jana NR, 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 EA, Olivieri M, Francavilla S, Gulino A, Ziparo E and D'Armiento M (1990) Ontogenesis of the nuclear 3,5,39-triiodothyronine receptor in the rat testis. *Endocrinology* 126:2521–2526.

Jannini EA, Dolci S, Ulisse S and Nikodem VM (1994) Developmental regulation of the thyroid hormone receptor α1 mRNA expression in the rat testis. *Mol Endocrinol* 8:89–96.

Jannini EA, Ulisse S and D'Armiento M (1995) Thyroid hormone and male gonadal function. *Endocr Rev* 16:443-459.

Jannini EA, Carosa E, Rucci N, Screponi E and D'Armiento M **(**1999) Ontogeny and regulation of variant thyroid hormone receptor isoforms in developing rat testis. *J Endocrinol Invest* 22:843– 848.

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

Jansen HT, Kirby JD, Cooke PS, Arambepola N and Iwamoto GA (2007) Impact of neonatal hypothyroidism on reproduction in the male hamster, *Mesocricetus auratus*. *Physiology and Behavior* 90:771–781.

Jean–Faucher C, Berger M, de Turckheim M, Veyssiere G and Jean C (1985) Testosterone and dihydrotestosterone levels in the epididymis, vas deferens and prepubertal gland of mice during sexual maturation. *Int J Androl* 8:44-57.

Jutte NHPM, Jansen R, Grootegoed JA, Rommerts FFG, Clausen OPF and van der Molen HJ (1982) Regulation of survival of rat pachytene spermatocytes by lactate supply from Sertoli cells. *J Reprod Fertil* 65:431-438.

King J (1974) 6-phosphogluconate dehydrogenase. In : Bergmeyer HG (ed) *Methods of Enzymatic Analysis*, Vol.2, pp 632-635, Academic Press, New York.

Laslett AL, Li LH, Jester WF Jr and Orth JM (2000) Thyroid hormone down-regulates neural cell adhesion molecule expression and affects the attachment of gonocytes in Sertoli cell-gonocyte co-cultures. *Endocrinology* 141:1633-164.

Löhr GW and Waller HD (1974) Glucose-6-phosphate dehydrogenase. In: Bergmeyer HU (ed) *Methods in Enzymatic Analysis*, Vol.2, pp. 636-643, Academic press, New York.

Longcope C (2000) The male and female reproductive system in hypothyroidism. In: Braverman LE and Utiger RD (eds), *Werner and Ingbar's The Thyroid*, 8th ed, pp 849-852, Lippincott-Raven Publishers, Philadelphia.

Macchia E, Nakai A, Janiga A, Sakurai A, Fisfalen ME, Gardner P, Soltani K and DeGroot LJ (1990) Characterization of site-specific polyclonal antibodies to c-erbA peptides recognizing human thyroid hormone receptors alpha 1, alpha 2, and beta and native 3,5,3'-triiodothyronine receptor, and study of tissue distribution of the antigen. *Endocrinology* 126: 3232-3239.

Manna PR, Kero J, Tena–Sempere M, Pakarinen P, Stocco DM and Huhtaniemi IT (2001) 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* 142:319-331.

Maran RRM (1997) *Modulatory effect of thyroid hormones on Leydig cell activity.* Ph.D. Thesis, University of Madras, Chennai, India.

Maran RRM, Ravisankar B, Ravichandran K, Valli G, Arunakran J and Aruldhas MM (1999a) Impact of neonatal onset hypothryroidism on Sertoli cell number, plasma and testicular interstitial fluid androgen binding protein. *Endocr Res* 25:307-322.

Maran RRM, Sivakumar R, Arunakaran J, Ravisankar B, Ravichandran K, Sidharthan V, Jeyaraj DA, Aruldhas MM (1999b) Duration-dependent effect of transient neonatal hypothyroidism on Sertoli and germ cell number and plasma and testicular interstitial fluid androgen binding protein concentration. *Endocr Res* 25:323-340.

Mayes PA (1988) Bioenergetics and the metabolism of carbohydrates and lipids. In: Murray RK, Granner DK, Mayes PA and Rodwell VW (eds) *Harper's Biochemistry*, 21st ed, pp 93-253, Appleton and Lange, California.

McCoard SA, Wise TH and Ford JJ (2003) Endocrine and molecular influence on testicular development in Meishan and White composite boars. *J Endocrinol* 178:405–416.

Means AR, Dedman JR, Tindall DJ and Welsh MJ (1978) Hormonal regulation of Sertoli cells. *Int J Androl* 2 (Suppl):403-423.

Mendis-Handagama SM and Ariyaratne HBS (2005) Leydig cells, thyroid hormones and steroidogenesis. *Indian J Exp Biol* 43: 939-962.

Myant NB (1964) The thyroid and reproduction in mammals. In: Pitt-Rivers R and Trotter WR (eds.), *The Thyroid Gland*, Vol I, pp 283-302, Butterworths, London.

Nunez J (1988) Mechanism of action of thyroid hormone. In: Cook BA, King RJB and van der Molen HJ (eds), *Hormones and their Actions*, Part I, pp 61-80, Elsevier Science Publishers, North Holland.

Ochoa S (1955). Malic enzyme in pigeon liver and wheat germ. *Methods Enzymol* I:739-741.

Oppenheimer JH, Schwartz HL and Surks MI (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 JH, Coulombe P, Schwartz HL and Gutfeld NW (1978) Nonlinear (amplified) relationship between nuclear occupancy by triiodothyronine and the appearance rate of hepatic alpha-glycerophosphate dehydrogenase and malic enzyme in the rat. *J Clin Invest* 61: 987-997.

Oppenheimer JH, Schwartz HL, Mariash CN, Kinlaw WB, Wong CW and Freake HC (1987) Advances in our understanding of thyroid hormone action at the cellular level. *Endocr Rev* 8:288-308.

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

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

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

Palmero S, Benahemad M, Morera AM, Trucchi P and Fugassa E (1992) Identification of nuclear tri-iodo thyronine receptors in Sertoli cells from immature piglet testis. *J Mol Endocrinol* 9:55-59.

Palmero S, Prati M, De Marco P, Trucchi P and Fugassa E (1993) Thyroidal regulation of nuclear tri-iodothyronine receptors in the developing rat testis. *J Endocrinol* 136:277–282.

Palmero S, De Marco P and Fugassa E (1995) Thyroid hormone receptor bmRNA expression in Sertoli cells isolated from prepubertal testis. *J Mol Endocrinol* 14:131–134.

Panno ML, Sisci D, Salerno M, Lanzino M, Pezzi V, Morrone EG, Mauro L, Palmero S, Fugassa E and Andó S (1996a) Thyroid hormone modulates androgen and estrogen receptor content in the Sertoli cells of peripubertal rats. *J Endocrinol* 148: 43-60.

Panno ML, Pezzi V, Salerno M, Palmero S, Fugassa E, Ando S, Sisci D and Lanzino M (1996b) Effect of triiodothyronine administration on estrogen receptor contents in peripuberal Sertoli cells. *Eur J Endocrinol* 134:633-638.

Rao GS, Cronrath CM and Rao ML (1984) Induction of malic enzyme in explants of human adipose tissue by L-3,5,3'-triiodothyronine. *Horm Metab Res* 14:16-20.

Rich KA and de Kretser DM (1983) Spermatogenesis and the Sertoli cell. In: de Kretser DM, Burger DH and Hudson B (eds), *The Pituitary and Testis*, pp 84-105, Springer-Verlag, Heidelberg .

Sharpe RM (1993) Experimental evidence for Sertoli-germ cell and Sertoli-Leydig cell interactions. In: Russell LD, Griswold MD (eds.), *The Sertoli Cell*, pp 391-418, Cache River Press, Clearwater, FL.

Sharpe RM, Mc Kinnell C, Kivlin C and Fisher JS (2005) Proliferation and functional maturation of Sertoli cells and their relevance to disorders of testis function in adulthood. *Reproduction* 125:769-784.

Simorangkir DR, Wreford NG and de Kretser DM (1997) Impaired germ cell development in the testes of immature rats with neonatal hypothyroidism. *J Androl* 18:186-193.

Skinner MK (1991). Cell-cell interactions in the testis. *Endocr Rev* 12:45-77.

Skinner MK (2005) Sertoli cell-somatic cell interactions. In: Skinner MK and Griswold MD (eds), *Sertoli Cell Biology*, pp 317-327, Elsevier Science, USA.

Skinner MK and Griswold MD (1980) Sertoli cells synthesize and secrete transferrin-like protein. *J Biol Chem* 255:9523-9525.

Smith S and Abraham S (1975) Fatty acid synthase from lactating rat mammary gland. *Methods Enzymol* XXXV:75-79.

Srere PA (1962) Citrate cleavage enzyme. *Methods Enzymol* V:641-644.

Steinberger A and Steinberger E (1971) Replication pattern of Sertoli cells in maturing rat testis *in vivo* and in organ culture. *Biol Reprod* 4:84-87.

Steinberger A, Heindel JJ, Lindsey JN, Elikington JSH, Sanburn BM and Steinberger E (1975) Isolation and culture of FSH responsive Sertoli cells. *Endocr Res Commun* 2:261-272.

Steiner AL, Pagliara AS, Chase LR and Kipnis DM (1972) Radioimmunoassay for cyclic nucleotides. II – Adenosine 3', 5'-monophosphate and guanosine 3', 5'-monophosphate in mammalian tissue and body fluids. *J Biol Chem* 247:1114-1120.

Sufi SB, Donaldson A and Jeeffcoat SL (1980) World Health Organization Center for Research Service in the Immunoassay of Hormones in Human Reproduction. *WHO Method Manual*, 10th Edition, pp 57-70, 85-99, WHO.

Swerdloff RS, Walsh PC, Jacobs HS and Odell WD (1971) Serum LH and FSH during sexual maturation in the male rat: effect of castration and cryptorchidism. *Endocrinology* 88:120-128.

Tagami T, Nakamura H, Sasaki S and Imura H (1990) Characterization of interaction between nuclear T_3 receptors and antiserum against cellular-erb A peptide. *Endocrinology* 126:1105-1111.

Ueno H and Mori H (1990) Morphometrical analysis of Sertoli cell ultrastructure during seminiferous epithelial cycle in rats. *Biol Reprod* 43:769-776.

Venkatesh NS (2004) *Temporal shift in adult rat testicular steroidogenesis towards estradiol due to transient neonatal hypothyroidism.* Ph.D. Thesis, University of Madras, Chennai, India.

White HB (1975) Glycerol phosphate dehydrogenase of chicken breast muscle. *Methods Enzymol* XLI:245-249.

Zar JH (1954) *Biostatistical Analysis*. Prentice Hall Inc., Englewood Cliff, New Jersey.