CHAPTER 6

Neonatal hypothyroidism retards body and ovarian growth and hampers adult ovarian functions in the rat

Thyroid hormones are required for normal body functions in mammals to control a variety of physiological processes. Hypothyroidism is a common disorder associated with a range of reproductive abnormalities including menstrual disorders, amenorrhea, infertility and frequent abortions in many female animals, including humans (Mochizuki, 1977; Louvet *et al.*, 1979; Bohnet *et al.*, 1981; Longscope, 1991; Maruo *et al.*, 1992a; Stradtman, 1993; Chan and Ng, 1995). In adult rats, hypothyroidism has been shown to affect normal follicular maturation of the ovary (Bruni *et al.*,1975; Hagino, 1971; Maruo *et al.*,1987; Armada-Dias *et al.*, 2001; Sato and Jiang, 2001) and gonadotropin secretion (Bruni *et al.*,1975), resulting in irregular estrous cycles (Mattheji *et al.*,1995; Hastuta *et al.*, 2004). Experimentally induced inadequate thyroid hormone supply has been reported to disturb folliculogenesis (Dijkstra *et al.*, 1996). It has also been shown that thyroidectomy before puberty increases the number of follicles (Tamura *et al.*, 1998a) and also a reduction in pre-ovulatory luteinizing hormone (LH) surge

(Tamura *et al.*, 1998b). Importance of thyroid hormones in embryonic or foetal development of vertebrates has also been well established (Sullivan *et al.*, 1987). Hypothyroidism in rats has also been shown to result in fewer pregnancies and reduction in litter size (Varma *et al.*, 1978; Hapon *et al.*, 2003). Dijkstra *et al.* (1996) in their study on prepubertal hypothyroidism induced by PTU feeding from birth to day 40 post partum had recorded decreased body and ovarian weight. They noted more secondary and less antral follicles, smaller non-atretic antral follicles and more atretic follicles. Even postnatal mice subjected to hypothyroidism have been shown to reduce number of Graffian follicles, and multi laminar follicles in the immediate post-treatment periods (Chan and Ng, 1995). There are also many other studies on the effect of PTU treatment during postnatal period, which have recorded retarded growth and physical development, delay in eye opening and teeth eruption (Meisami, 1984; Tamasy *et al.*,1984; Kawada *et al.*,1988; Akaike *et al.*,1991; Madeira *et al.*,1991, 1992; Akaike and Kato, 1997). However, long-term consequences of neonatal hypothyroidism in the pre-weaning period in terms of adult ovarian functions and hormonal profile have not been clearly elucidated. It is in this context that the present study on rat neonates subjected to hypothyroidism in terms of their ovarian functions and hormonal titres in the adult age has been carried out.

MATERIAL AND METHODS:

Animals and Maintenance:

Healthy female laboratory rat neonates (Charles foster strain) were used for the present study. The animals were maintained at Sarabhai Research Center, with a constant temperature range of 21 ±2°C and a lighting regimen of LD 8:16 throughout the experimental period of study. The animals were fed with standard diet (Amrut Rat Feed) and water *ad libitum*. The treatment was initiated on day '0' (day of birth) and terminated on day 21 postpartum.

Experimental Protocol:

The experimental setup was divided into two groups of study.

Group I: Control (C)

Female rat neonates were maintained in the laboratory till day 90 and served as controls. This consisted of 2 subgroups (as follows) of 10 animals each:

(i) Control rats (Maintained as such)

(ii) Injected *i.p.* with vehicle (0.9% saline) in evening at 16.30 hrs.

Group II: Hypothyroid (HPOT)

Female rat neonates consisting of 10 animals were subjected to transient hypothyroidism (HPOT) by feeding mothers with 0.1% 6-propyl 2-thiouracil (PTU) (procured from Sigma Chemical Co. USA) in drinking water from day 0 to day 21 post partum.

Parameters and Methods of Evaluation:

As in chapter 5

Histology and Histometry:

As in chapter 5

Hormone Assays:

As in chapter 5

STATISTICAL ANALYSIS:

All data are expressed as mean ± SEM. The data were analyzed by student's 't' test and analysis of variance (ANOVA) wherever applicable, at 95% confidence limit.

RESULTS:

Since there was no significant difference between the values of the two subgroups of controls, the data of only vehicle control is considered.

Body and Ovary weight:

The body weight of hypothyroid rats was significantly lower at all ages from 22 days to 90 days. The final weight at 90 days was 36% lesser than the controls (144.5 ±2.55 gm v/s 226.16±9.57 gm) (Table 6.1; Figs. 6.1a, 6.1b, 6.1c). Correspondingly the growth rate was also consistently low. The absolute and relative weights of paired ovaries were also significantly less in hypothyroid rats. The absolute weight was almost 50% lesser than the controls (Table 6.2; Figs. 6.2a, 6.2b).

Table 6.1: Chronological alteration showing body weight, absolute and relative ovary weight of control and hypothyroid female rats

Treatment	Body weight			Absolute Ovary weight			Relative Ovary weight		
	Age in Days			Age in Days			Age in Days		
	22	45	90	22	45	90	22	45	90
C	51.33 ±1.873	136.667 ±4.176	226.167 ±9.575	0.024 ±0.0003	0.062 ±0.0069	0.076 ±0.0074	0.046 ±0.0006	0.045 ±0.0015	0.034 ±0.0025
HPOT	27.00[c] ±0.447	70.667[c] ±2.512	144.5[c] ±2.552	0.014[c] ±0.0004	0.017[c] ±0.0016	0.039[b] ±0.0066	0.051[c] ±0.0016	0.025[c] ±0.0021	0.027[b] ±0.0044

C – Control; **HPOT** – Hypothyroid

Values expressed as Mean ± SEM of ten animals; [a] $p<0.01$; [b] $p<0.005$, [c] $p<0.0005$

Table 6.2: Per day Body and Ovary Growth Rate (g/day) in Control and Hypothyroid female rats

Treatment	Per Day Body Growth Rate			Per Day Ovary Growth Rate		
	Age in days			Age in days		
	0-22	22-45	45-90	0-22	22-45	45-90
C	2.055	1.896	1.000	0.00009	0.00084	0.00015
HPOT	0.995	0.970	0.820	0.00007	0.00018	0.0002

C – Control; HPOT – Hypothyroid

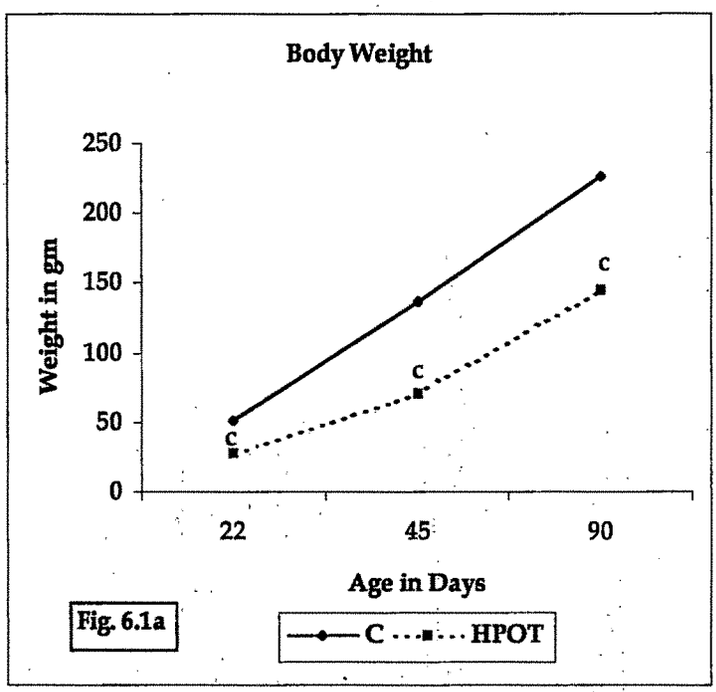


Fig. 6.1a

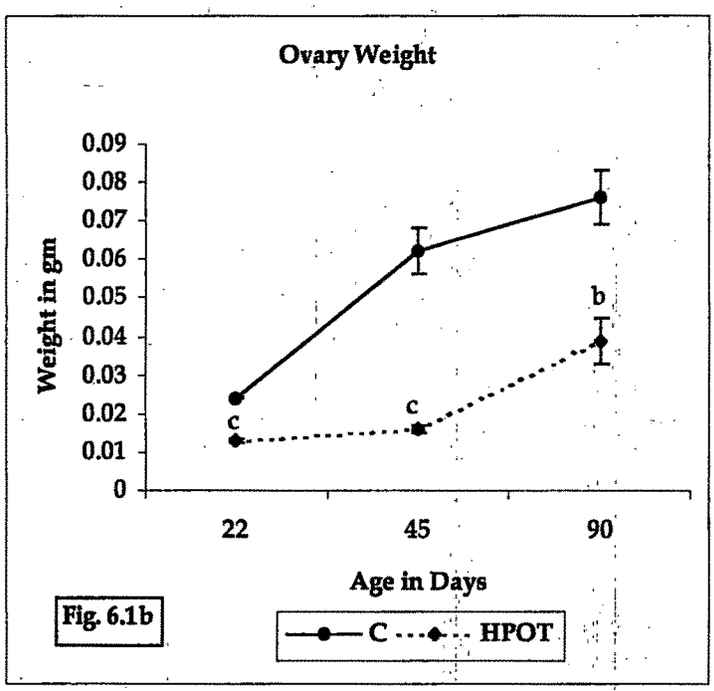


Fig. 6.1b

Figures 6.1a&b: Chronological alterations showing body and ovary (g) weight in control (C) and hypothyroid (HPOT) rats

Values expressed as Mean ± SEM of ten animals;

$^{a}p<0.01$; $^{b}p<0.005$, $^{c}p<0.0005$

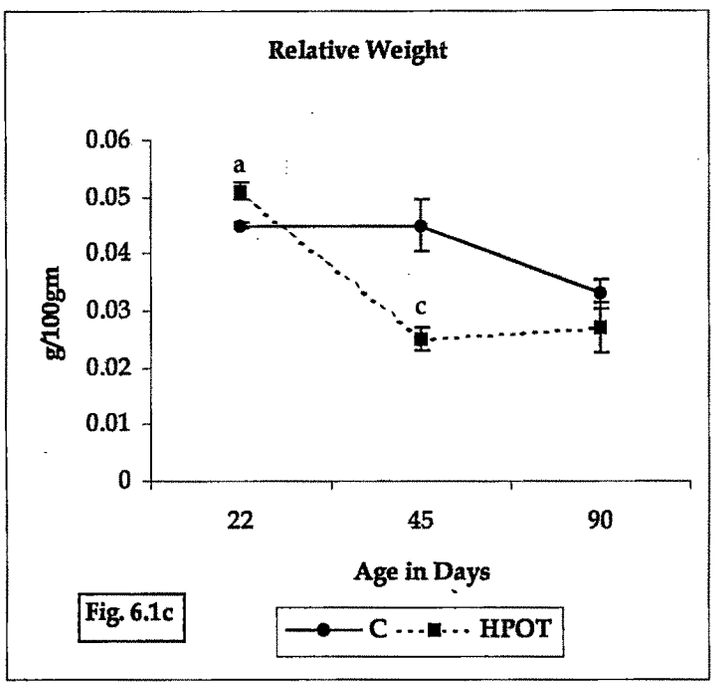
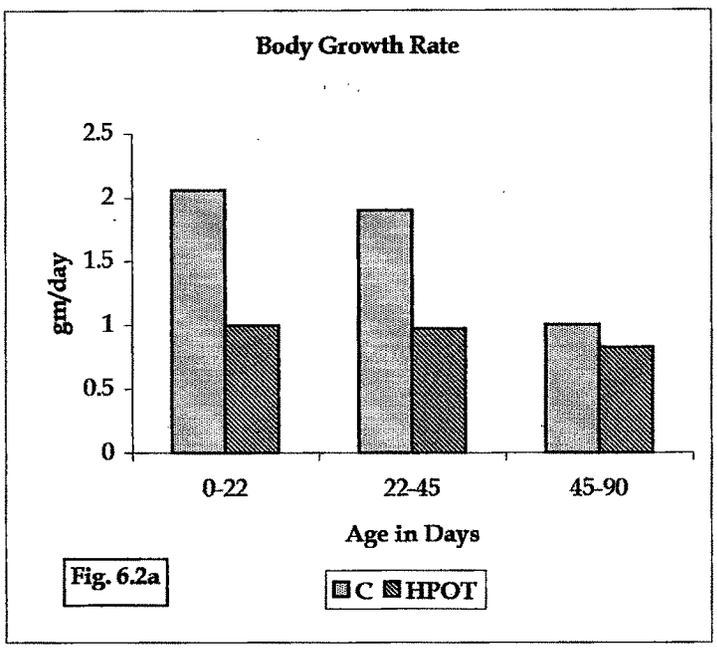
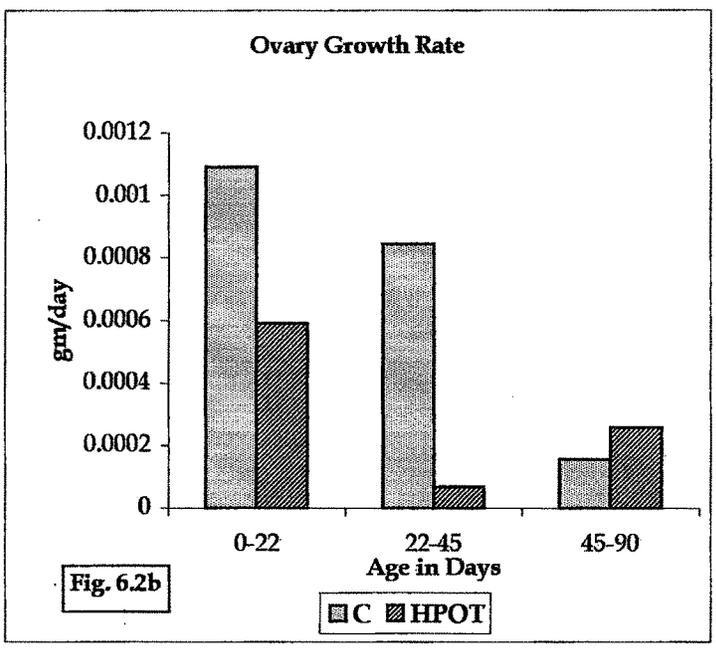


Figure 6.c: Chronological alterations showing relative (g/100g) ovary weight in control (C) and hypothyroid (HPOT) rats

Values expressed as Mean ± SEM of ten animals;

$^{a}p<0.01$; $^{b}p<0.005$, $^{c}p<0.0005$





Figures 6.2a&b: Chronological alterations showing body and ovary (g/day) growth rate in control (C) and hypothyroid (HPOT) rats

Ovarian Histology and Histometry:

The ovary of HPOT rats showed significantly lower volume at all ages with reduced number of follicles of all developmental grades (Plate VIA and VIB). Though follicles of all developmental stages showed significant reduction in HPOT rats, the most remarkable changes were with reference to antral follicles, which were very low and atretic follicles, which were very high (Table 6.3).

Table 6.3: Differential total follicular count in ovary of control and hypothyroid female rats

Treatment	Age in Days	Follicle Type						Ovarian Volume (mm^3)
		Primordial	Primary	Secondary	Antral	CL	Atretic	
C	22	821 ±48	531 ±25	350 ±20	181 ±04	-	10 ±0.8	0.58
	45	426 ±17	162 ±11	245 ±10	142 ±08	-	29 ±02	0.68
	90	300 ±13	168 ±08	96 ±04	72 ±05	48 ±05	36 ±03	1.31
HPOT	22	523 [c] ±39	354 [c] ±31	296 ±21	38 [c] ±07	-	247 [c] ±23	0.23 [c]
	45	339 ±31	146 ±13	192 ±19	56 [c] ±05	-	321 [c] ±16	0.36 [c]
	90	251 ±22	83 [c] ±06	72 ±10	18 [c] ±02	33 ±03	336 [c] ±27	0.85 [c]

C – Control; HPOT – Hypothyroid

Values expressed as Mean ± SEM of ten animals; [a] $p<0.01$; [b] $p<0.005$, [c] $p<0.0005$

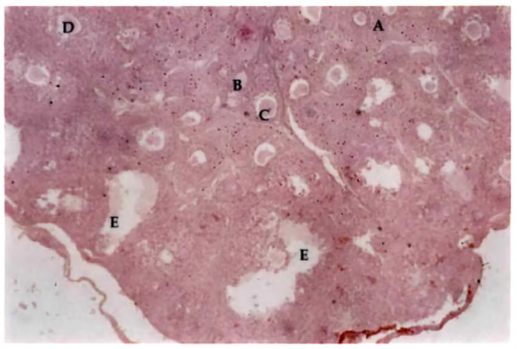
PLATE - VIA

Figures: **Photomicrographs of sections of Ovary of Control and hypothyroid rats**

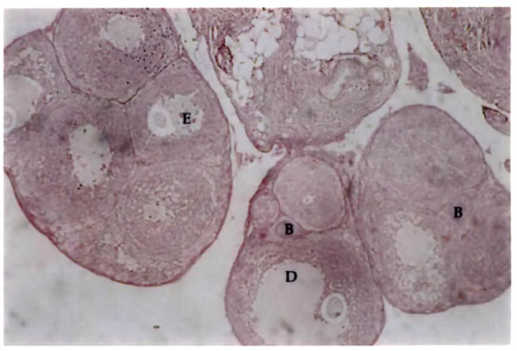
Sections of ovaries of 22 and 45 day old control rats showing less number of atretic follicles compared to age-matched hypothyroid rats that shows more number of atretic follicles and poorly developed follicles.

A - Primordial follicle, B - Primary follicle, C - Secondary follicle, D - Antral follicle, E - Atretic follicle, GC - Granulosa cells, TC - Thecal cells, OT - Oocyte

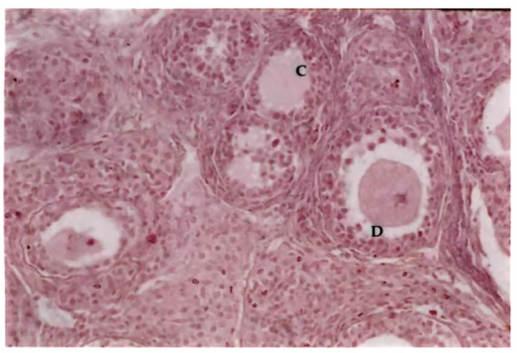
PLATE - VIA



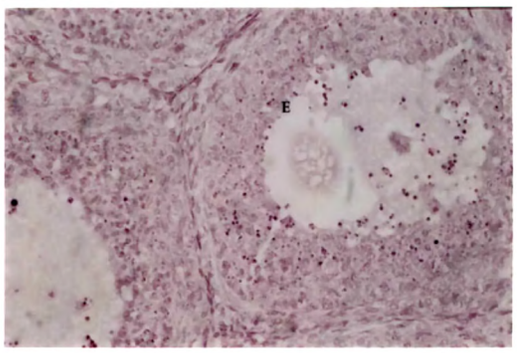
C 22 10x



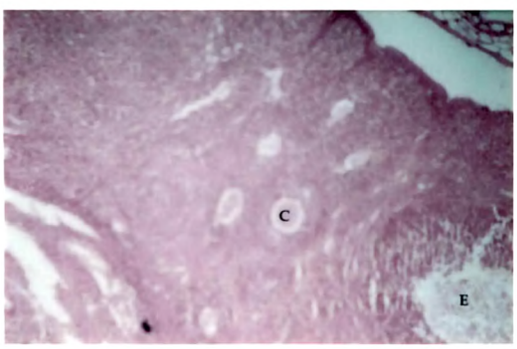
HPOT 22 10x



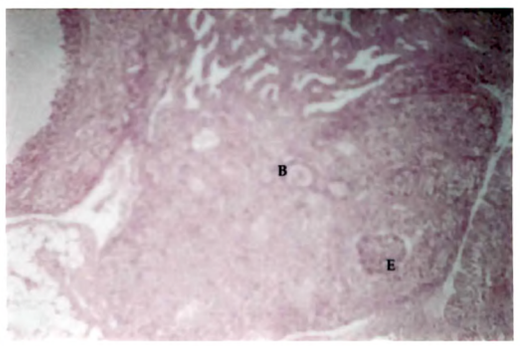
C 22 40x



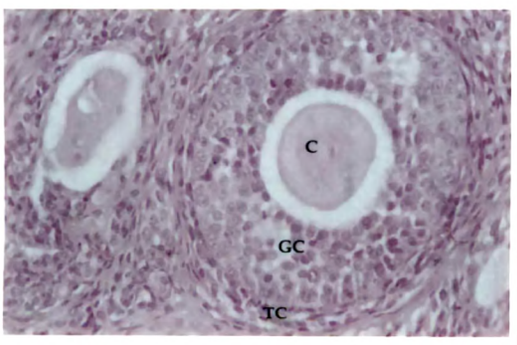
HPOT 22 40x



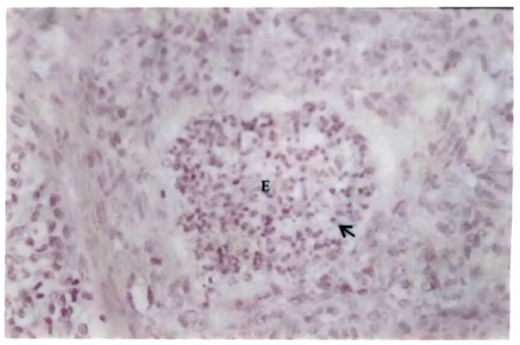
C 45 10x



HPOT 45 10x



C 45 40x



HPOT 45 40x

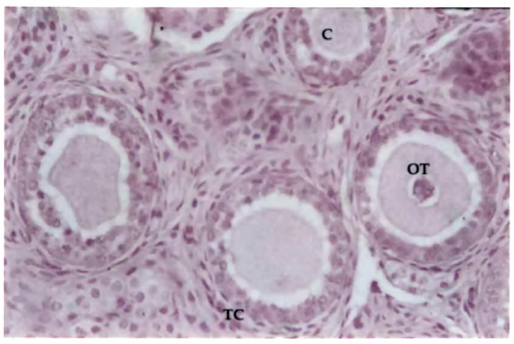
PLATE – VIB

Figures: **Photomicrographs of sections of Ovary of Control and hypothyroid rats**

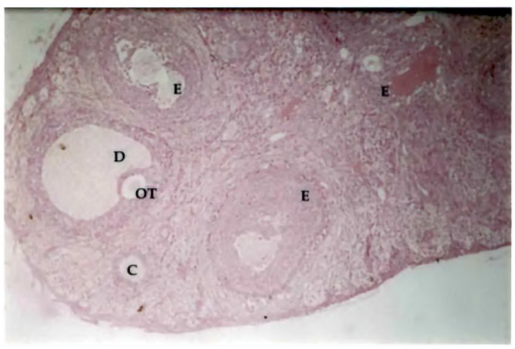
Sections of ovaries of 90 day old control rats showing less number of atretic follicles compared to lower volume with reduced number of follicles in hypothyroid rats that shows more number of atretic follicles.

A – Primordial follicle, B – Primary follicle, C - Secondary follicle, D – Antral follicle, E – Atretic follicle, GC – Granulosa cells, TC – Thecal cells, OT – Oocyte

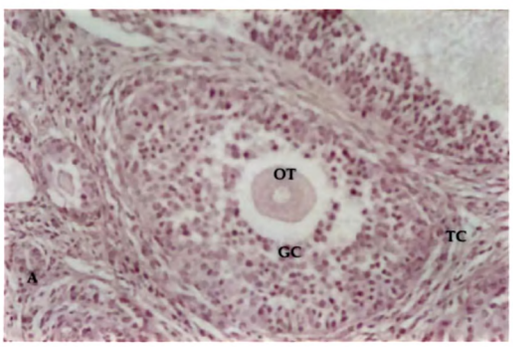
PLATE - VIB



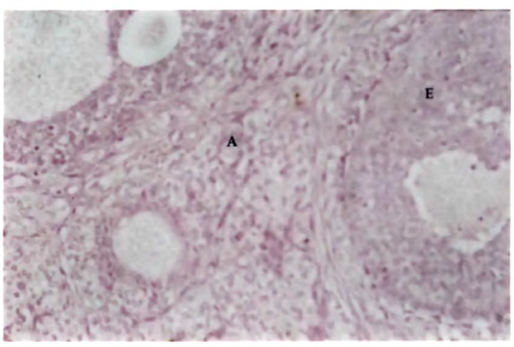
C 90 10x



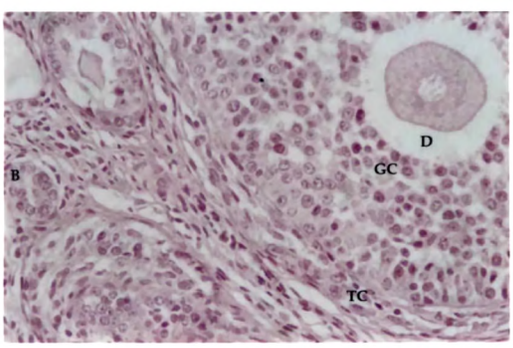
HPOT 90 10x



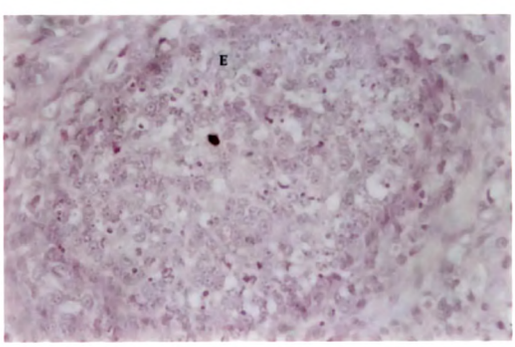
C 90 25x



HPOT 90 25x



C 90 40x



HPOT 90 40x

Serum Hormone profiles:

The circulating titres of both T_3 and T_4 were significantly elevated at all ages in HPOT rats than controls. On the other hand, estrogen and progesterone showed significant reduction at all ages post-treatment (Tables 6.4 & 6.5; Figs. 6.4a, 6.4b, 6.5a, 6.5b). In order to avoid the contradiction due to differential levels of estrogen and progesterone during the estrous cycle, the serum levels of only those animals, which were in late diestrous or early proestrous on the day of sacrifice, were assayed and the values represented are on an average of 3-6 animals.

Table 6.4: Serum hormone profiles of T3 and T4 (ng/ml) in control and melatonin treated female rats

Treatment	T3			T4		
	Age in Days			Age in Days		
	22	45	90	22	45	90
C	0.251 ±0.051	0.303 ±0.107	0.653 ±0.053	0.38 ±0.013	1.170 ±0.061	2.368 ±0.255
HPOT	0.444[b] ±0.018	0.843[c] ±0.004	0.557 ±0.108	5.257[c] ±0.37	3.05[c] ±0.081	2.13 ±0.09

C - Control; HPOT - Hypothyroid

Values expressed as Mean ± SEM of ten animals;

[a]p<0.01; [b]p<0.005, [c]p<0.0005

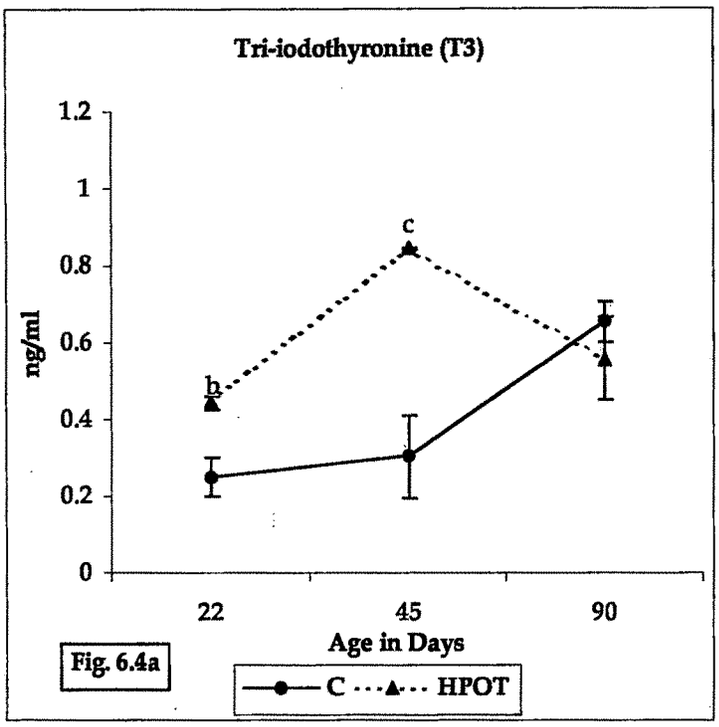
Table 6.5: Serum hormone profiles of Estradiol and Progesterone (ng/ml) in control and melatonin treated female rats

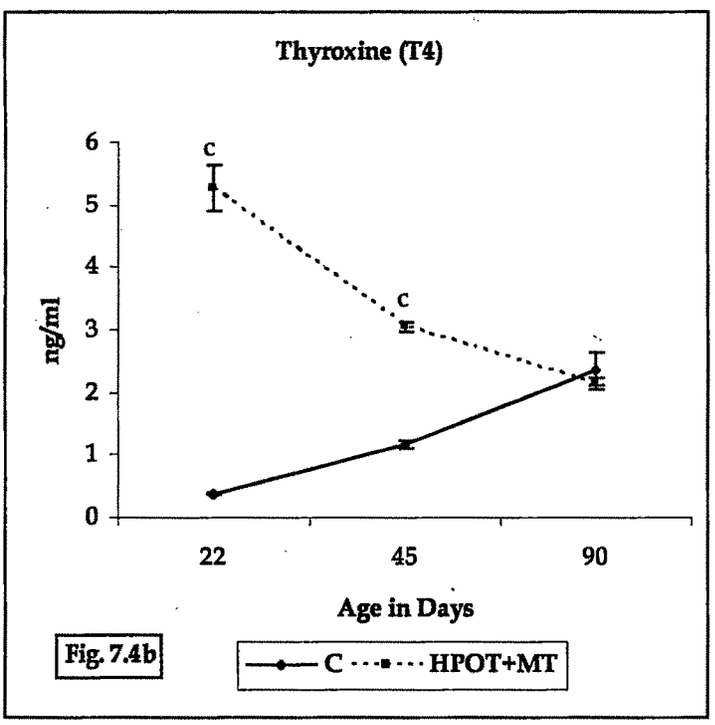
Treatment	Estradiol			Progesterone		
	Age in Days			Age in Days		
	22	45	90	22	45	90
C	4.02 ±1.30	8.73 ±2.55	10.39 ±3.10	2.75 ±0.88	8.55 ±2.41	15.19 ±4.36
HPOT	3.01 ±0.94	5.68 ±1.89	7.81 ±2.96	3.809 ±0.356	2.392 ±0.313	7.601 ±3.238

C - Control; HPOT - Hypothyroid

Values expressed as Mean ± SEM of 3-6 animals;

[a]p<0.01; [b] p<0.005, [c]p<0.0005

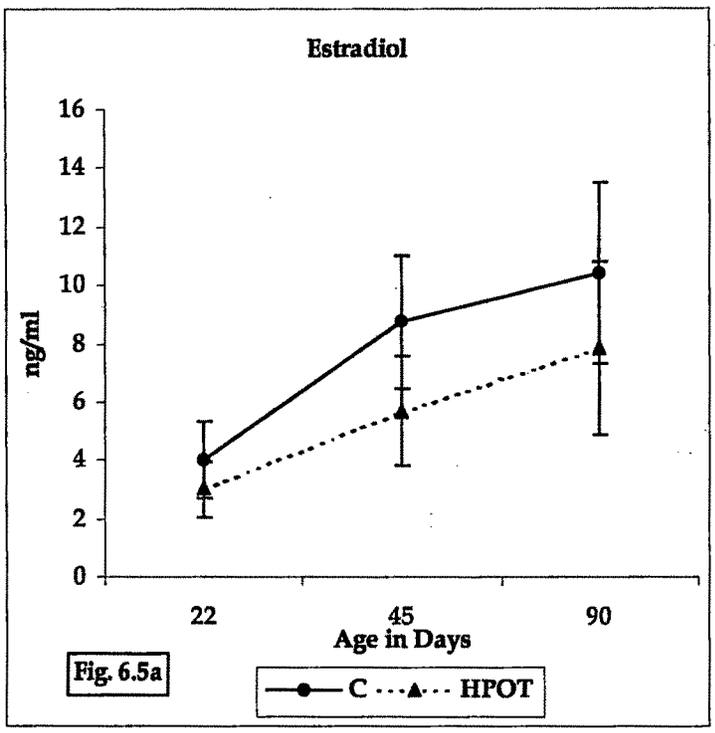


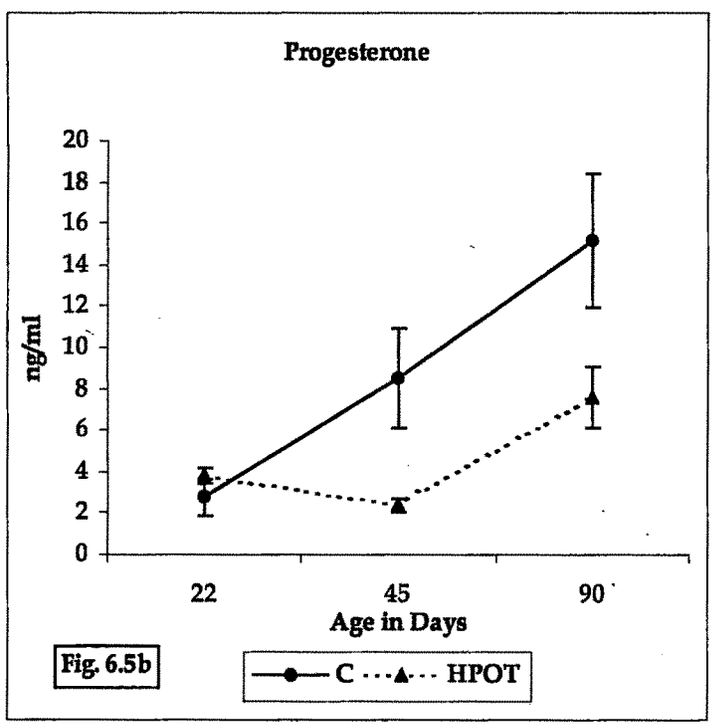


Figures 6.4a&b: Chronological alterations showing serum T3 and T4 (ng/ml) levels in control (C) and hypothyroid (HPOT) rats

Values expressed as Mean ± SEM of ten animals;

$^{a}p<0.01$; $^{b}p<0.005$, $^{c}p<0.0005$





Figures 6.5a&b: Chronological alterations showing serum Estradiol and Progesterone (ng/ml) levels in control (C) and hypothyroid (HPOT) rats

Values expressed as Mean ± SEM of 3-6 animals;

$^{a}p<0.01$; $^{b}p<0.005$, $^{c}p<0.0005$

DISCUSSION:

The results of the present study on PTU induced hypothyroidism from the day of birth till 21 days reveal that neonatal hypothyroidism has significant retardative effect on body weight gain and ovary growth and, ovarian function marked by follicular dynamics and serum hormone profiles. Though there are many reports on prenatal or postnatal hypothyroidism induced reproductive disturbances, the present study is the only one, which shows a clear long-term negative influence of hypothyroidism on folliculogenesis, body weight gain and adult hormonal profiles. The presently recorded poor growth rate and significantly low adult body weight are well correlated by a previous observation of reduced body weight in rats rendered hypothyroidic from day 0 day 25 (Zertashia *et al.*, 2002). They also showed a reduction in ovarian weight. A clear strain difference in the degree of hypothyroidism induced decrement in body and ovarian weight is evident as in the present study with Charles foster strain of rats a decrement in body weight by 36% and in ovarian weight by 49% has been recorded as against 19.8% and 26.1% respectively with Sprague-Dawley strain of rats by Zertashia *et al.* (2002).

This strain difference in the degree of response is also manifest in follicular dynamics as Zertashia *et al.* (2002) did not observe any difference in the mean number of various types of follicles, while,

the present study clearly shows a reduction in the number of various non-atretic follicles though, the total number of follicles was not very different compared to those of control animals. This is explainable by the variation in the percentage of atretic follicles. Whereas the ovaries of control animals showed only 5% atretic follicles, those of HPOT animals show as much as 42.3% at 90 days. It is inferable that there is no difference in folliculogenesis on a quantitative basis; there is nevertheless a decrease in the number of developing follicles essentially due to significantly higher degree of atresia. Apparently, neonatal hypothyroidism does influence adult ovarian functions on a long-term basis by down-regulating follicular survival and up-regulating follicular apoptosis.

This relationship between hypothyroidism and follicular apoptosis is strengthened by the recent report of Singh *et al.* (2003) of hypothyroidism during development down regulating the anti-apoptotic genes and maintaining a high level of the pro-apoptotic gene, Bax, in the cerebellum. Jiang *et al.* (2002) had also documented hampered folliculogenesis due to hypothyroidism and further improvement in folliculogenesis and estradiol secretion by thyroxine administration (Jiang *et al.*, 2000). Prepubertal hypothyroidism has also been shown to hamper differentiation of granulosa cells with consequent generation of antral follicles of smaller diameter (Dijkstra *et al.*, 1996).

The present study also reveals decreased estrogen production and increased progesterone production as a long-term effect of 90 days due to neonatal hypothyroidism. Since conversion of progesterone to estrogen by the follicles requires the expression of aromatase activity under the influence of FSH, the possibility of neonatal hypothyroidism permanently down-regulating either FSH action or aromatase activity need to be evaluated. Some supportive evidence can be drawn towards this by the reported expression of thyroid hormone receptor mRNA in oocytes, granulosa and cumulus cells (Maruo *et al.*, 1992a; Zhang *et al.*, 1997) and the reported ability of thyroid hormone to affect oocyte maturation, aromatase activity, estradiol secretion and functional differentiation of granulosa (Wakim *et al.*, 1995a, b; Dijkstra *et al.*, 1996; Gregaroszik *et al.*, 1998; Cecconi *et al.*, 1999). A recent study by Tohei (2004) has shown dysfunction in gonadal axis at the hypothalamic-pituitary level in male rat and inhibition of follicular development accompanied by decreased estradiol secretion and increased progesterone in female rats by hypothyroidism. Since this increase in plasma progesterone concentration due to hypothyroidism in adult female rats has been attributed to a hyposecretion of prolactin (Tohei *et al.*, 1998), the likelihood of permanent resetting towards increased prolactin secretion due to neonatal hypothyroidism also needs to be investigated.

From the present results it can be concluded that the hypothyroidism in the postnatal phase has serious effects on adult ovarian functions involving folliculogenesis, atresia and ovarian hormone output. These effects may be centrally mediated as well as by altered local regulatory mechanisms.

SUMMARY:

The present investigation has evaluated the long-term effects of neonatal hypothyroidism (HPOT) on adult ovarian functions, histoarchitechture and, serum hormone profiles in Charles foster strain of rats. HPOT was induced in neonates through lactating mothers by feeding 0.1% 6-propyl 2-thiouracil in drinking water from day 0 to day 21. The animals were assessed at 22, 45 and 90 days of age in terms of body and ovary weights, ovarian histometry and serum titres of T3, T4, estradiol and progesterone. There was significant decrease in adult body and ovary weights in HPOT animals than that of controls and significant decrease in ovarian volume and reduced number of follicles. The number of antral follicles showed a significant decrement and the number of atretic follicles were high. From these findings, it can be concluded that neonatal HPOT has a long-term deleterious effect on adult ovarian functions in terms of growth, maturation, folliculogenesis and reduced serum titres of estradiol and progesterone.