CHAPTER 4

Simultaneous corticosterone administration to neonates subjected to light induced functional pinealectomy prevents the adverse effects on early germ cell survival but not on spermatids and sperms in adult testis

Pre and postnatal sexual maturation depend on a temporal series of factors. Apart from the amount of androgens present in certain phases of development, environmental stressors are quite relevant in influencing reproductive maturation (Biagini and Pich, 2002). The hypothalamo-hypophyseal-adrenal (HPA) axis is the main modulator of stress responses in mammals and this axis is generally regarded as immature and insensitive in the foetal period (Sapolsky and Meaney, 1986; De Kloet *et al.*, 1987). Further a blunted stress response has been described in newborn rats and this so called "stress hypo-responsive period" (SHRP) was found to be extending up to the second week of life in rodents (see Hennessy, 1997; Vazquez, 1998). This period is characterized by low basal levels of ACTH, corticosterone and also of testosterone. This would suggest that the testes, which were very active immediately before birth (Orth *et al.*, 1983; Ward and Weisz, 1980) have become silent. Steroidogenesis by testes recommences in rats again at puberty, 3-6 weeks after birth, when histochemical 3β

HSDH activity reappears in the Leydig cells (Niemi and Ikonen, 1962). The initial period of life, in which the newborn begins to adapt itself to the environment, is therefore characterized by low steroid hormone titres. Nevertheless, there is evidence to suggest that a stress stimulus applied during the SHRP could result in permanent behavioural changes manifested in the adult animals (Hennessy, 1997; Vazguez, 1998). It was also shown by Biagini et al. (1998) that rat pups subjected to a maternal separation stress during the first week of life, manifested an increased stress response by prolonged corticosterone release to mild stressors in the adulthood, mainly due to decreased sensitivity to corticosterone feedback regulation.

Since pre-natally stressed rats are hyper-responsive to stress stimuli and also show changes in development of male reproductive function (Weinstock, 1997; Reznikov *et al.*, 1999). Neonatal exposure to stress also could show important alterations in the maturation of the sexual phenotype as consequent changes affecting the entire HPA axis (Schmidt *et al.*, 2004). Significant behavioural changes occur consequent to maternal separation stress and could be related with the increased glucocorticoid secretion (Biagini and Pich, 2002). Rat pups treated with corticosterone in the first week of life presented long-lasting changes in hippocampal glucocorticoid receptor (GR) expression (Zoli *et al.*, 1990) similar to the changes noted in maternally separated rats (Biagini *et al.*, 1998). The dysregulation of HPA axis

neonates subjected to LFPx in terms of body and testis weights, adult testis functions and hormonal profiles. An attempt is also made to assess the time dependent effect of corticosterone by administration in the morning ($CORT_M$) or in the evening ($CORT_E$).

MATERIALS AND METHODS:

Animals and Maintenance:

Healthy male 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 under a lighting regimen of LD 8:16 or LD 24:0 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 five groups of six animals each.

Group I: Control (C)

Male rat neonates were maintained under normal lighting regimen of LD 8:16 and were provided with food and water *ad libitum*.

Group II and III: Vehicle Controls:

Male rat neonates were functionally pinealectomised by exposure to continuous light from day 0 to day 21 and simultaneously administered with vehicle (0.9% saline) in the morning at 06.00 hrs and in the evening at 18.00 hrs respectively.

Group IV and V: Functional Pinealectomy and Corticosterone treatment (LFPx+CORT):

Male Rat neonates were functionally pinealectomised by exposing them to continuous light from day '0' to day '21' and simultaneously administered CORT (1μg /animal/day from day 0 to 10 and 2 μg from day 11 to 21) in the evening ($LFPx+CORT_E$) at 18.00 hrs and (2μg /animal/day from day 0 to 10 and 4 μg from day 11 to 21) ($LFPx+CORT_M$) in the morning at 06.00 hrs.

Parameters and Methods of Evaluation:

As in chapter one

Histology and Histometry:

As in chapter one

Hormone Assays:

As in chapter one

STATISTICAL ANALYSIS:

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

RESULTS:

Since there was no significant difference between normal control and vehicle control, only data of normal control is taken into consideration.

Body and Testes weight

The adult body weight at 90 days of LFPx+CORT animals was significantly less relative to control rats. The relative weight of testis at 90 days was not significantly different in LFPx+$CORT_M$ or LFPx+$CORT_E$ rats compared to controls, though initially in the pre-pubertal ages there was significant decrease (Tables 4.1 & 4.2; Figs. 4.1a, 4.1b, 4.1c, 4.1d, 4.1e, 4.1f and 4.2a, 4.2b, 4.2c, 4.2d).

Table 4.1: **Chronological alterations showing body weight (g), absolute (g) and relative (g/100g) testes weight of control and functionally pinealectomized corticosterone treated male rats**

Treatment	Body Weight				Absolute Testes Weight				Testes Relative Weight			
	Age in Days				Age in Days				Age in Days			
	35	45	60	90	35	45	60	90	35	45	60	90
C	96.00 ±3.804	123.00 ±3.677	197.00 ±6.396	361.00 ±6.280	0.860 ±0.050	1.350 ±0.058	2.600 ±0.085	3.301 ±0.104	0.890 ±0.033	1.10 ±0.027	1.33 ±0.033	0.92 ±0.030
LFPx+CORT$_M$	72^c ±1.753	154^c ±2.136	265^c ±3.497	341^a ±2.955	0.21^c ±0.010	1.30 ±0.039	2.86^b ±0.045	3.18 ±0.036	0.29^c ±0.007	0.85^c ±0.023	1.08^c ±0.022	0.93 ±0.013
LFPx+CORT$_E$	98 ±3.219	153^c ±2.349	228^b ±9.680	337^a ±10.249	0.59^c ±0.023	1.30 ±0.064	2.12^c ±0.074	3.22 ±0.059	0.588^c ±0.017	0.842^c ±0.031	0.933^c ±0.018	0.961^c ±0.014
LFPx*	108 ±6.396	157^c ±2.456	268^c ±7.496	348 ±16.730	0.94 ±0.051	1.36 ±0.030	2.67 ±0.085	3.11 ±0.140	0.87 ±0.038	0.87^c ±0.006	1.00^c ±0.017	0.90 ±0.024
CORT$_M$@	115.5^c 0.671	173.6^c ±1.763	267.8^c ±3.301	315.8^a ±3.270	1.069 ±0.009	1.81 0.056	2.79 ±0.032	3.01 ±0.050	0.998^a ±0.079	1.003 ±0.082	1.031^a ±0.076	0.983^c ±0.083
CORT$_E$@	109.167^a ±4.053	160.833^c ±3.439	250.5^c ±4.945	332.667 ±10.582	0.984 ±0.047	1.805^c ±0.063	2.309 ±0.085	3.243 ±0.067	0.904 ±0.044	1.128 ±0.057	0.921^c ±0.089	0.967^c ±0.020

C – Control; LFPx+CORT$_M$- Functionally Pinealectomized and morning corticosterone; LFPx+CORT$_E$- Functionally Pinealectomized and evening corticosterone; LFPx – Functionally Pinealectomized (Continuous Light); CORT$_M$ – Morning Corticosterone; CORT$_E$ – Evening Corticosterone

* - Values taken from Chapter 1

@ - Values taken from Bhavsar, (2001)

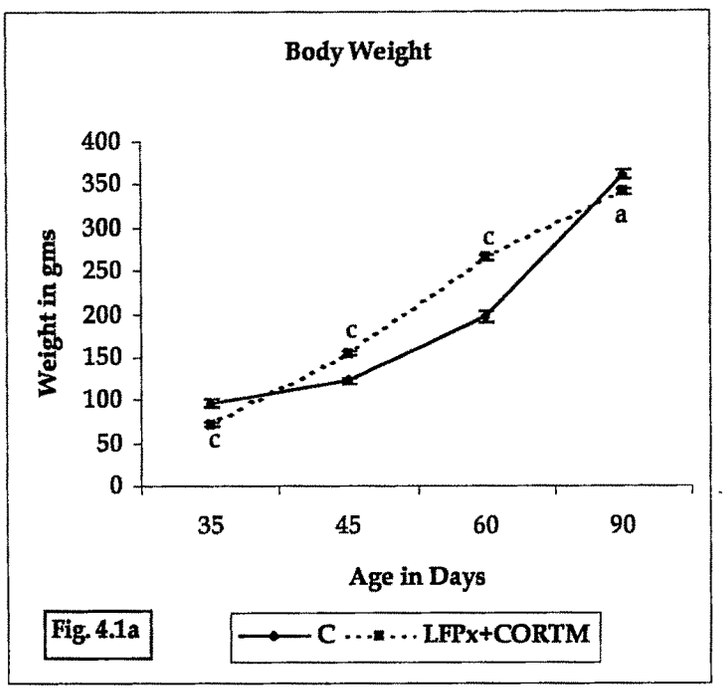
Table 4.2: Per day Body and Testes Growth Rate (g/day) in Control and Functionally pinealectomised Corticosterone treated rats.

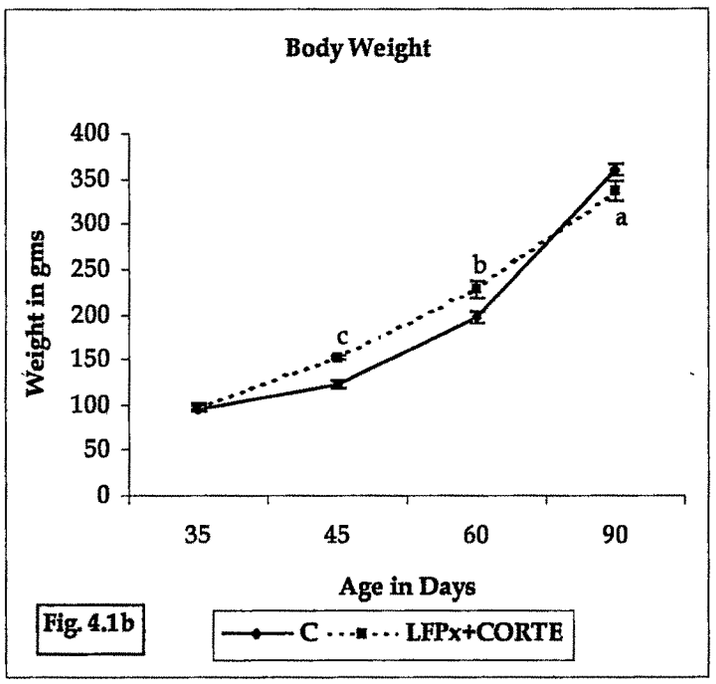
Treatment	Per Day Body Growth Rate				Per Day Testes Growth Rate			
	Age in days				Age in days			
	0-35	35-45	45-60	60-90	0-35	35-45	45-60	60-90
C	2.583	0.589	1.247	1.815	0.024	0.011	0.021	0.008
LFPx+$CORT_M$	1.88	1.82	1.856	0.843	0.006	0.024	0.026	0.004
LFPx+$CORT_E$	2.625	1.215	1.264	1.204	0.017	0.016	0.014	0.012
LFPx*	2.934	1.07	1.863	0.888	0.027	0.009	0.022	0.005
$CORT_M$@	3.11	5.81	6.28	1.60	0.029	0.074	0.065	0.0073
$CORT_E$@	2.92	5.167	5.978	2.739	0.027	0.082	0.034	0.031

C – Control; LFPx+$CORT_M$- Functionally Pinealectomized and morning corticosterone; LFPx+$CORT_E$- Functionally Pinealectomized and evening corticosterone; LFPx – Functionally Pinealectomized (Continuous Light); $CORT_M$ – Morning Corticosterone; $CORT_E$ – Evening Corticosterone

* - Values taken from Chapter 1

@ - Values taken from Bhavsar, (2001)





Figures 4.1a&b: Chronological alterations showing body weights (g) of control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

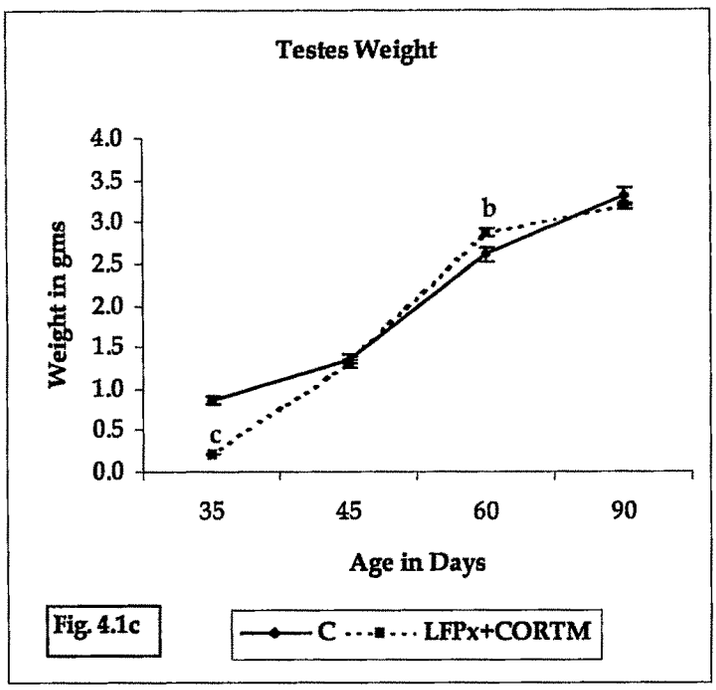


Fig. 4.1c

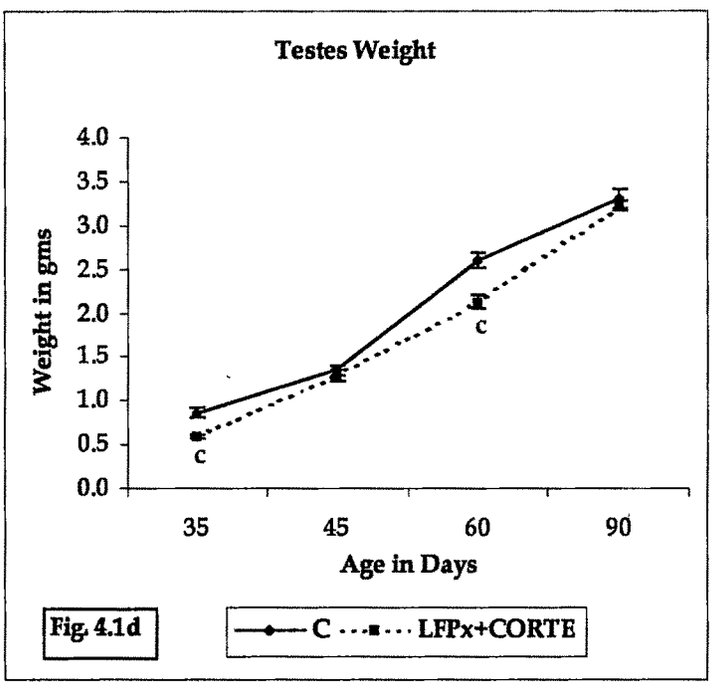


Fig. 4.1d

Figures 4.1c&d: Chronological alterations showing testes (g) weight of control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

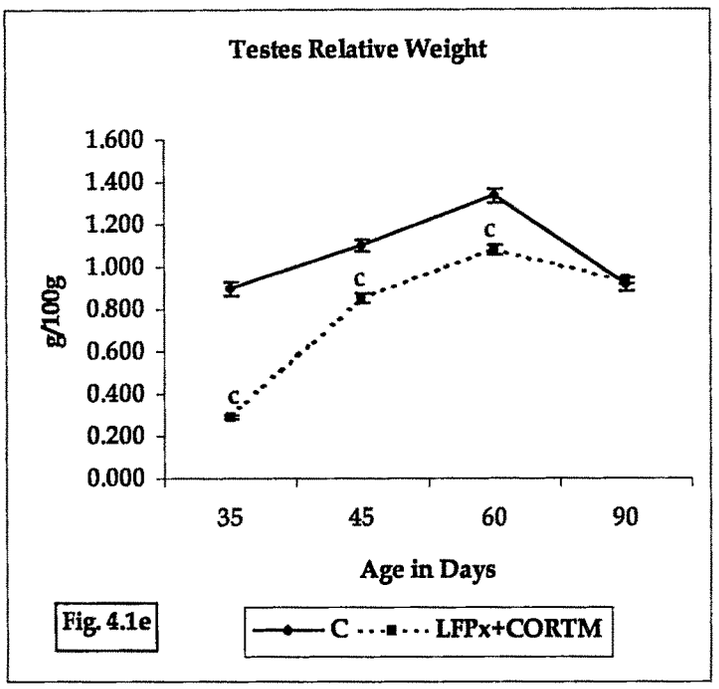


Fig. 4.1e

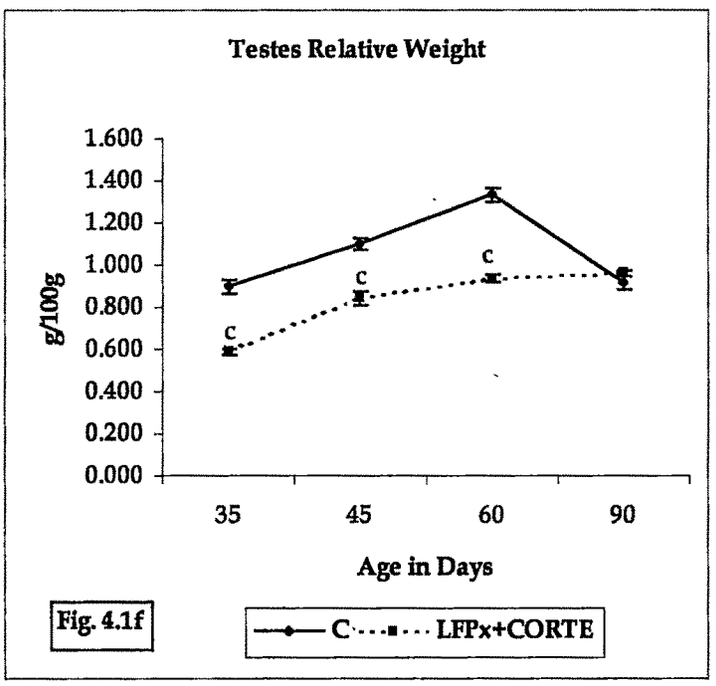
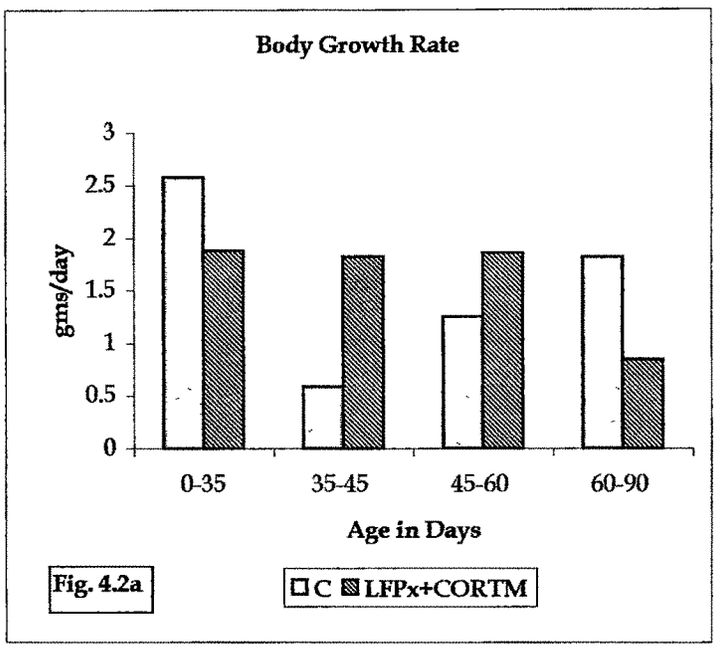


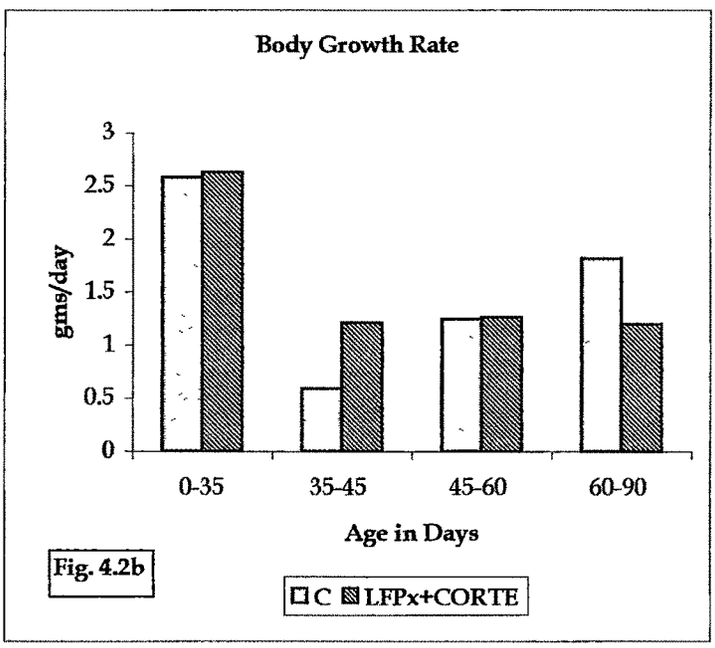
Fig. 4.1f

Figures 4.1e&f: Chronological alterations showing testes (g/100g) relative weight of control (C) and functionally pinealectomised corticosterone (morning and evening) treated (LFPx+$CORT_M$) and (LFPx+$CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

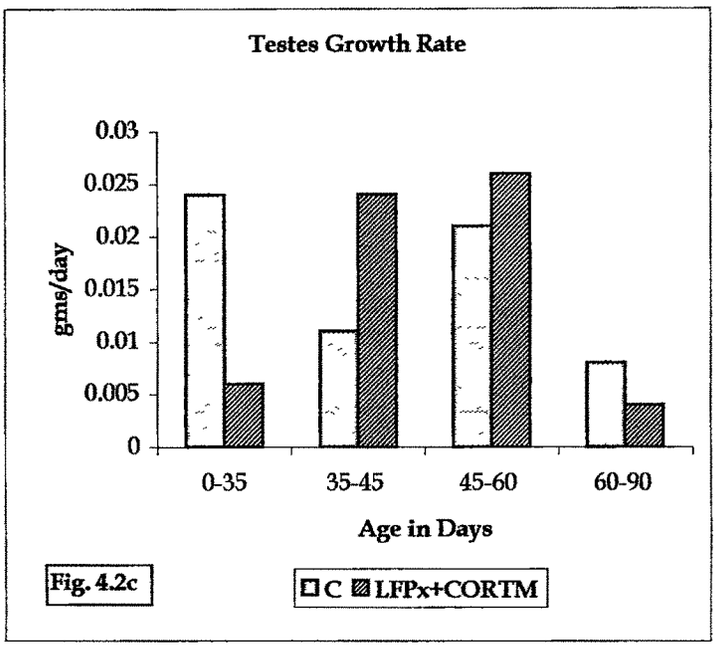


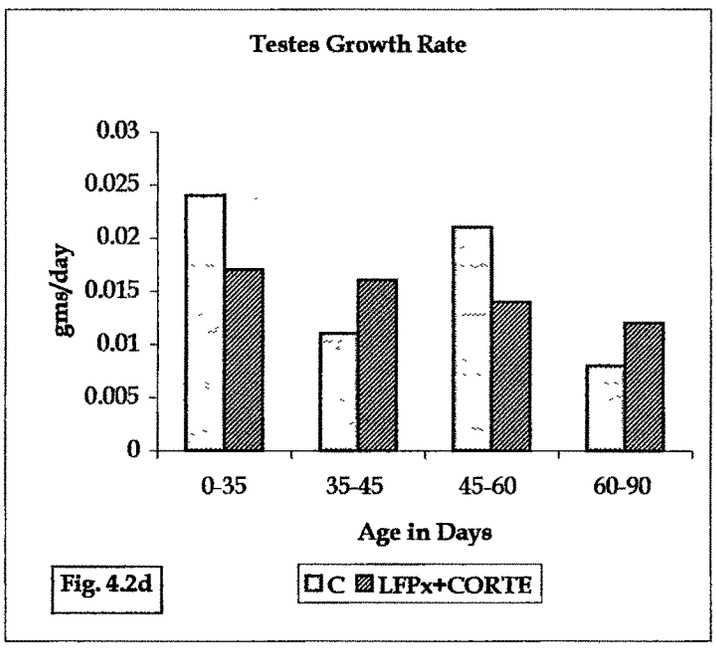


Figures 4.2a&b: Chronological alterations showing body (g/day) growth rate of control (C) and functionally pinealectomised corticosterone (morning and evening) treated (LFPx+$CORT_M$) and (LFPx+$CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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





Figures 4.2c&d: Chronological alterations showing testes (g/day) growth rate of control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

Histology and Histometrics of Testis

Control:

At 35 days well formed seminiferous tubules with meiotic germ cells and well-formed interstitial cells could be seen. Spermatogenesis progressed to elongating spermatids by 45 days. By 60 days, tubules were well formed and spermatogenesis was fully established with sperms seen in most of the tubules. The Leydig cells were well formed. Fully established spermatogenesis in tubules and well formed Leydig cells were the features at 90 days (Plate IA).

LFPx+CORT$_M$:

Though closely packed small tubules could be seen at 35 days, the basement membrane was greatly thickened and many germ cells were vacuolated and even some cells showed pycnotic nuclei. By 45 days, tubules were enlarged and spermatogenesis had progressed up to round spermatids. Prominent interstitium with relatively more number of Leydig cells could be seen. At 60 days, the testis showed closely packed tubules with fully established spermatogenesis but most of the tubule showed quantitatively less number of sperms. The 90day testis was also characterized by closely packed tubules with more number of early stages of germ cells and with prominent Leydig cells. However, there were less number of sperms in the tubules (Plate IVA).

LFPx+CORT$_E$:

At 35 days the testis showed well-formed tubules with thickly populated germ cells. The Leydig cells in the interstitium were less prominent. By 45 days, the closely packed enlarged tubules showed progression of spermatogenesis up to elongating spermatids. At 60 days, the tubules were found closely packed with very little interstitial space and with thickly populated germ cells. The tubules continued to be closely packed with very little interstitial space and germ cells up to elongating spermatids; however there were very little sperms. Even at 90 days, the condition remained more or less the same with very little sperms (Plate IVB).

Histometrics of Testis

There was no significant difference in testis and seminiferous tubular volume or total number of Sertoli cells between control and experimental rats of 90days. However, germinal epithelial cell thickness, tubular length, basement membrane area and total number of germ cells were significantly greater in experimental rats. The number of germ cells per meter length of tubules was significantly less in experimental rats (Table 4.3).

Table 4.3: Histometric enumeration of seminiferous tubules of Control, Functionally pinealectomised Corticosterone treated and corticosterone treated rats at 90 days

Treatment	T_V in cc	S_D in cm	GE in cm	S_V in cc	S_L in cm	bm in cm^2	SC_N $\times 10^6$	TGC_T $\times 10^6$	AGC_T $\times 10^6$	TGC_M $\times 10^6$	AGC_M $\times 10^6$	% Loss
C	1.503 ±0.030	0.0279 ±0.0006	0.0074 ±0.0003	1.427 ±0.050	2321.03 ±94.200	204.04 ±5.230	32.49 ±1.800	311 ±6.300	280.84 ±5.600	13.39 ±0.260	12.1 ±0.150	10.00 ±0.0002
LFPx+$CORT_M$	1.451 ±0.035	0.0235 ±0.0005	0.0116 ±0.0001	1.379 ±0.070	3170.59 ±76.785	234.47 ±6.590	31.01 ±0.745	330.88 ±3.405	14.05 ±0.135	267.88 ±1.995	11.79 ±0.172	11.08 ±0.119
LFPx+$CORT_E$	1.470 ±0.025	0.0236 ±0.0005	0.0114 ±0.0007	1.396 ±0.035	3181.26 ±92.950	236.45 ±7.585	31.87 ±0.596	316.13 ±2.652	11.33 ±0.635	245.29 ±1.386	8.05 ±0.148	9.108 ±0.338
LFPx*	1.419 ±0.055	0.0269 ±0.004	0.0192 ±0.0001	1.348 ±0.065	2357.96 ±58.260	199.81 ±4.350	35.22 ±0.106	330.83 ±3.405	267.88 ±1.995	14.05 ±0.135	11.79 ±0.172	14.18 ±0.119
$CORT_M$@	1.374 ±0.040	0.0338 ±0.009	0.0079 ±0.0003	1.305 ±0.060	2474.22 ±85.300	201.476 ±3.000	34.630 ±2.100	309.00 ±5.600	277.00 ±5.200	12.480 ±0.490	11.19 ±0.658	10.300 ±0.580
$CORT_E$@	1.775 ±0.160	0.0310^b ±0.0007	0.0100^c ±0.0002	1.668 ±0.150	2214.16 ±95.200	215.44^a ±3.250	30.998 ±1.500	416.00^c ±4.200	407.24^c ±6.800	18.78^c ±0.300	18.39^c 0.350	2.07^c ±0.300

C – Control; **LFPx+$CORT_M$** - Functionally Pinealectomized and morning corticosterone; **LFPx+$CORT_E$** - Functionally Pinealectomized and evening corticosterone; **LFPx** – Functionally Pinealectomized (Continuous Light); **$CORT_M$** – Morning Corticosterone; **$CORT_E$** – Evening Corticosterone;

Values expressed as Mean ± SEM of minimum fifteen observations. $^a p < 0.05$, $^b p < 0.005$, $^c p < 0.0005$

* - Values taken from Chapter 1; @ - Values taken from Bhavsar, (2001);

T_V - Volume of Testis, S_D - Seminiferous tubule diameter, **GE** - Germinal epithelial thickness, S_V - Volume of Seminiferous tubule, S_L - Length of seminiferous tubule, **bm** - basement membrane area of the seminiferous tubule, SC_N - Total Sertoli cell number in testis, TGC_T - Theoretical germ cell number per testis, AGC_T - Actual germ cell number per testis, TGC_M - Theoretical germ cell number per meter of seminiferous tubule, AGC_M - Actual germ cell number per meter of seminiferous tubule.

PLATE - IVA

Figures 1 - 8: **Photomicrographs of sections of testis of LFPx rats treated with Corticosterone in morning.**

Figures 1 and 2: Sections of testis of 35 day old rats showing closely packed tubules with thickened basement membrane with few pyknotic cells.

Figures 3 and 4: Section of testis of 45 day old rats of showing enlarged tubules. Spermatogenesis progressed up to round spermatids with prominent interstitium.

Figures 5 and 6: Section of testis of 60 day old rats showing closely packed tubules with fully established spermatogenesis and quantitatively less no. of sperms.

Figures 7 and 8: 90 day old testis section showing closely packed tubules with early stages of germ cells, prominent interstitium. Retarded spermatogenesis with very few sperms.

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Figures: 2, 4, 6, & 8 - 400 x

L - Lumen, S - Sperms, St - Spermatids, I - Interstitium

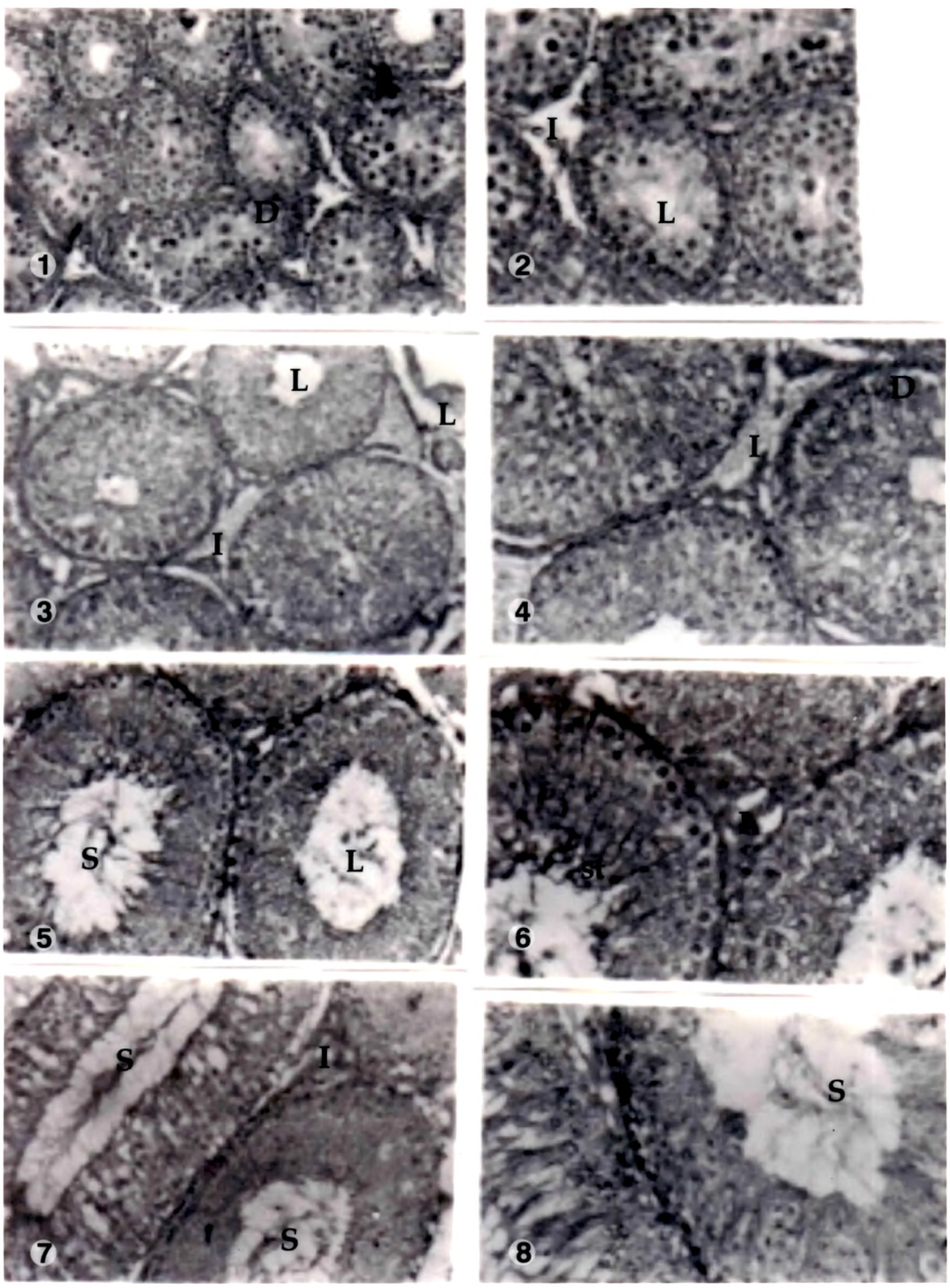

PLATE - IVA

Photomicrographs of Testis of Control & Functionally Pinealectomised Corticosterone Treated Rats

PLATE - IVB

Figures 1 - 8: **Photomicrographs of sections of testis of LFPx rats treated with Corticosterone in evening.**

Figures 1 and 2: Sections of testis of 35 day old rats showing well formed tubules with thick population of germ cells. Interstitium less prominent.

Figures 3 and 4: Section of testis of 45 day old rats of showing closely packed elongated tubules showing spermatogenesis up to elongating spermatids and thick population of germ cells with very little interstitial space.

Figures 5 and 6: Section of testis of 60 day old rats showing closely packed tubules with elongating spermatids and very few sperms.

Figures 7 and 8: 90 day old testis section showing retarded spermatogenesis with very few sperms.

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Figures: 2, 4, 6, & 8 - 400 x

L - Lumen, S - Sperms, St - Spermatids, I - Interstitium

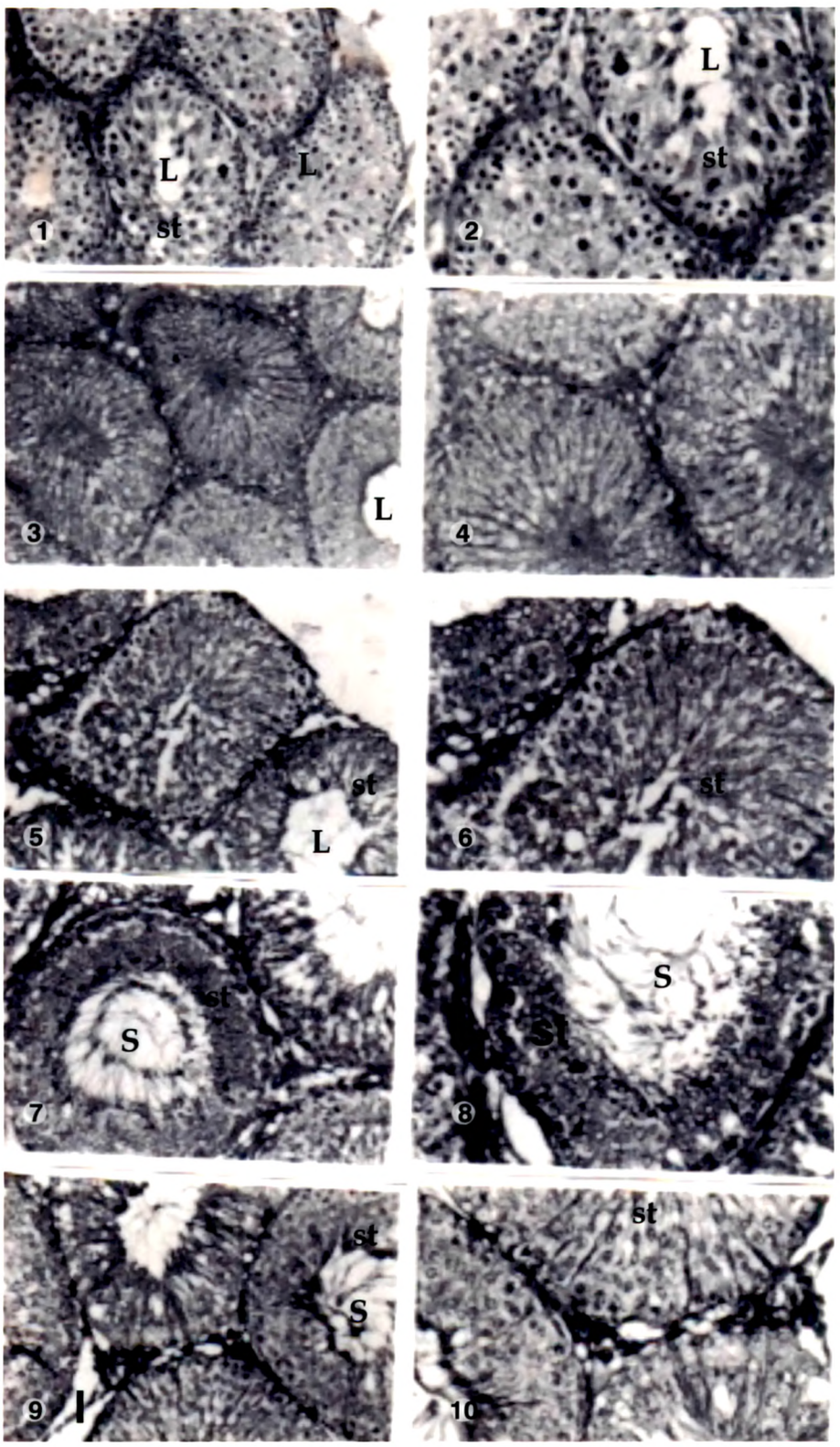


PLATE - IVB

Photomicrographs of Testis of Control & Functionally Pinealectomised Corticosterone Treated Rats

Serum Hormone Profile

TSH and T4 levels were higher from pubertal age onwards (45 days) in LFPx+$CORT_M$ rats. In contrast, the T4 levels were significantly lesser in adult ages (60 and 90 days). The adult T3 levels were significantly lesser in both LFPx+$CORT_M$ and LFPx+$CORT_E$ rats.

Serum LH and T levels were significantly less in both experimental groups at all ages except at 35 days whereas the serum corticosterone titres were significantly higher in both the experimental groups at all ages except for 45 day it was identical in LFPx+$CORT_E$ animals compared to control animals (Tables 4.4 & 4.5; Figs. 4.4a, 4.4b, 4.4c, 4.4d, 4.4e, 4.4f, 4.5a, 4.5b, 4.5c, 4.5d, 4.5e, 4.5f).

Table 4.4: Serum TSH, T_4 and T_3 (ng/ml) levels of Control and Functionally pinealectomised Corticosterone treated rats at 90 days

Treatment	TSH				T_3				T_4			
	Age in days				Age in days				Age in days			
	35	45	60	90	35	45	60	90	35	45	60	90
C	6.60 ±0.12	6.87 ±0.11	7.49 ±0.14	5.44 ±0.06	0.45 ±0.01	0.30 ±0.10	0.60 ±0.08	0.65 ±0.05	0.58 ±0.08	1.17 ±0.06	2.56 ±0.02	2.36 ±0.22
LFPx+$CORT_M$	5.97^b ±0.113	13.91^c ±0.067	8.88^c ±0.114	7.19^c ±0.057	1.22^c ±0.035	5.95^c ±0.121	2.75^c ±0.019	2.96^c ±0.186	0.443 ±0.095	0.332^c ±0.082	0.801^c ±0.074	0.394^c ±0.066
LFPx+$CORT_E$	12.79^c ±0.161	13.11^c ±0.085	11.11^c ±0.097	9.45^c ±0.113	3.20^c ±0.064	3.25^c ±0.019	2.09^c ±0.021	1.40^b ±0.177	0.44 ±0.081	0.55^c ±0.026	0.56^c ±0.022	0.32^c ±0.077
LFPx*	13.3^c ±0.06	11.6^c ±0.06	9.2^c ±0.13	6.81^c ±0.04	0.91^c ±0.01	0.55^a ±0.02	0.71 ±0.02	0.79^b ±0.01	3.00^c ±0.07	2.72^c ±0.02	3.09^c ±0.01	2.90^a ±0.02
$CORT_M$@	6.90 ±0.10	5.90^c ±0.09	7.1^a ±0.12	7.5^c ±0.18	1.4^c ±0.04	1.18 ±0.09	1.73^c ±0.069	2.48 ±0.094	1.6^c ±0.038	0.7^b ±0.017	0.8^a ±0.021	0.83^a ±0.043
$CORT_E$@	6.170^a ±0.144	7.283^a ±0.176	7.633 ±0.062	7.823^c ±0.125	0.985 ±0.112	1.230 ±0.182	1.650^c ±0.074	2.468 ±0.225	0.583^b ±0.031	0.734^c ±0.086	1.053^b ±0.056	0.400^b ±0.082

C – Control; LFPx+$CORT_M$- Functionally Pinealectomized and morning corticosterone; LFPx+$CORT_E$- Functionally Pinealectomized and evening corticosterone; LFPx – Functionally Pinealectomized (Continuous Light); $CORT_M$ – Morning Corticosterone; $CORT_E$ – Evening Corticosterone

Values expressed as Mean ± SEM of four samples. $^a p < 0.05$, $^b p < 0.005$, $^c p < 0.0005$

*- Values taken from Chapter 1; @ - Values taken from Bhavsar, (2001)

Table 4.5: Serum LH, Corticosterone and Testosterone (ng/ml) levels of Control and Functionally pinealectomised Corticosterone rats at 90 days

Treatment	LH				Corticosterone				Testosterone			
	Age in days				Age in days				Age in days			
	35	45	60	90	35	45	60	90	35	45	60	90
C	16.45 ±0.634	21.75 ±0.854	48.12 ±1.235	53.25 ±1.031	0.80 ±0.618	1.00 ±0.155	4.80 ±0.705	4.50 ±1.699	0.56 ±0.166	2.23 ±0.278	2.62 ±0.217	4.37 ±0.265
LFPx+CORT$_M$	8.83^c ±0.019	12.6^c ±1.021	9.21^c ±0.985	7.65^c ±0.772	2.40^a ±0.541	6.00^b ±1.223	0.65^b ±0.046	1.03^a ±0.052	2.20^c ±0.021	1.20^b ±0.131	2.10^b ±0.063	1.50^c ±0.024
LFPx+CORT$_E$	15.62 ±0.617	9.33^c ±0.017	13.53^c ±0.993	20.44^c ±0.782	2.47^a ±0.473	1.00 ±0.227	5.70 ±1.022	8.14^a ±0.882	1.30^b ±0.113	1.40^a ±0.162	1.70^b ±0.058	1.40^c ±0.153
LFPx*	5.48^c ±0.02	20.69 ±0.01	39.51^b ±0.01	23.02^c ±0.07	2.08^a ±0.02	3.50^c ±0.03	2.17^b ±0.01	1.48 ±0.01	6.60^c ±0.01	6.20^c ±0.02	5.90^c ±0.04	5.60^b ±0.03
CORT$_M$@	21.9^a ±2.61	13.15^b ±2.01	31.44^c ±1.78	31.3^c ±1.64	28.8^c ±2.80	21.0^c ±1.76	18.7^c ±1.065	15.6^c ±1.76	2.40^c ±0.099	1.60^a ±0.076	2.80 ±0.085	1.30^c ±0.032
CORT$_E$@	20.575^b ±0.776	10.650^c ±1.028	14.000^c ±1.135	26.875^c ±0.527	15.600^c ±0.208	13.245^c ±0.132	10.045^c ±0.522	9.100^c ±0.227	1.600^b ±0.122	1.550^a ±0.155	1.100^c ±0.041	1.000^b ±0.082

C – Control; LFPx+CORT$_M$- Functionally Pinealectomized and morning corticosterone; LFPx+CORT$_E$- Functionally Pinealectomized and evening corticosterone; LFPx – Functionally Pinealectomized (Continuous Light); CORT$_M$ – Morning Corticosterone; CORT$_E$ – Evening Corticosterone

Values expressed as Mean ± SEM of four samples. $^a p < 0.05$, $^b p < 0.005$, $^c p < 0.0005$

*- Values taken from Chapter 1; @ - Values taken from Bhavsar, (2001)

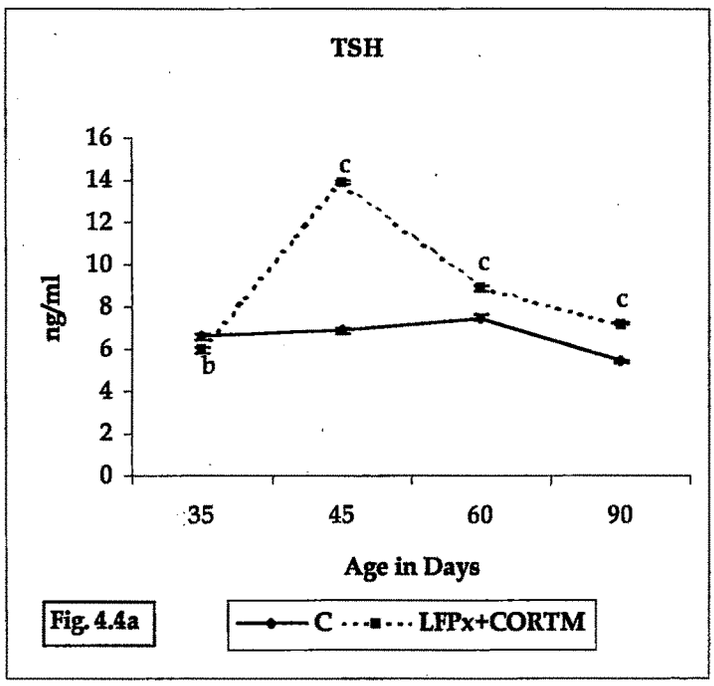


Fig. 4.4a

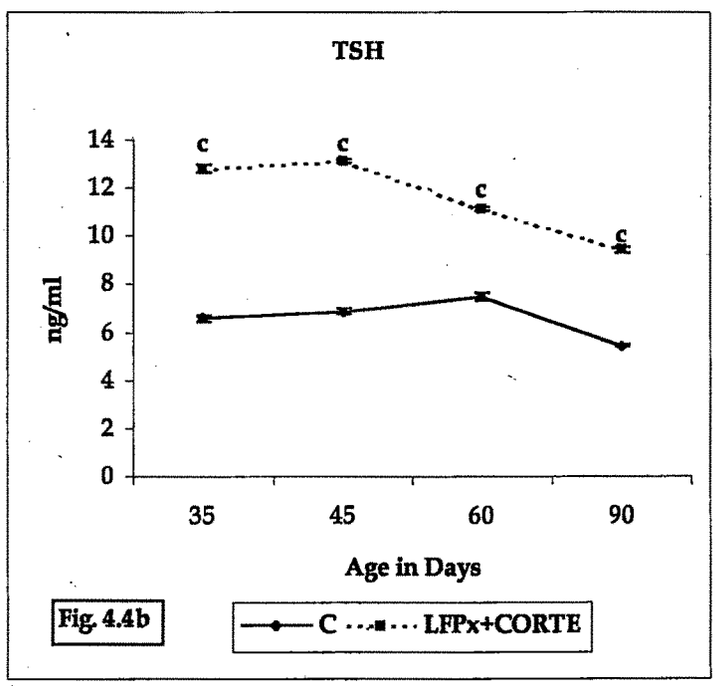


Fig. 4.4b

Figures 4.4a&b: Chronological alterations showing serum TSH (ng/ml) levels in control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

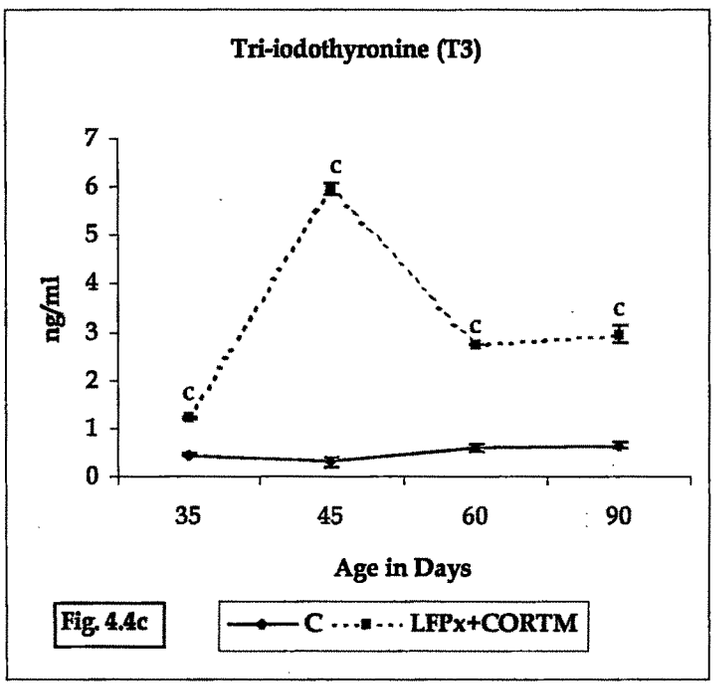


Fig. 4.4c

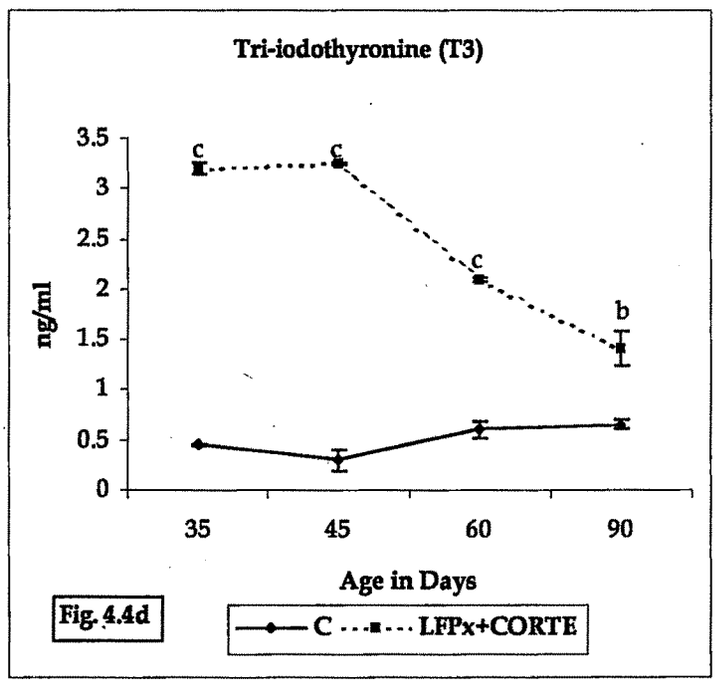


Fig. 4.4d

Figures 4.4c&d: Chronological alterations showing serum T3 (ng/ml) levels in control (C) and functionally pinealectomised corticosterone (morning and evening) treated (LFPx+$CORT_M$) and (LFPx+$CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

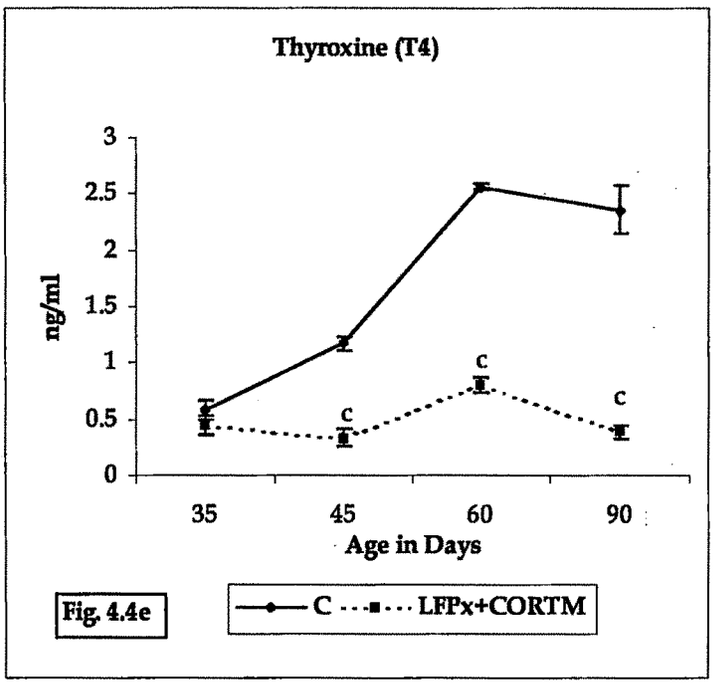


Fig. 4.4e

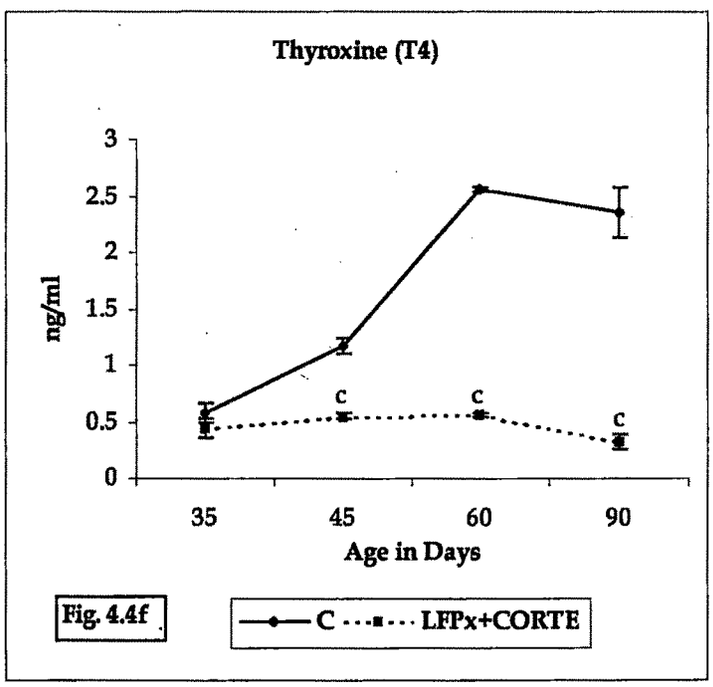
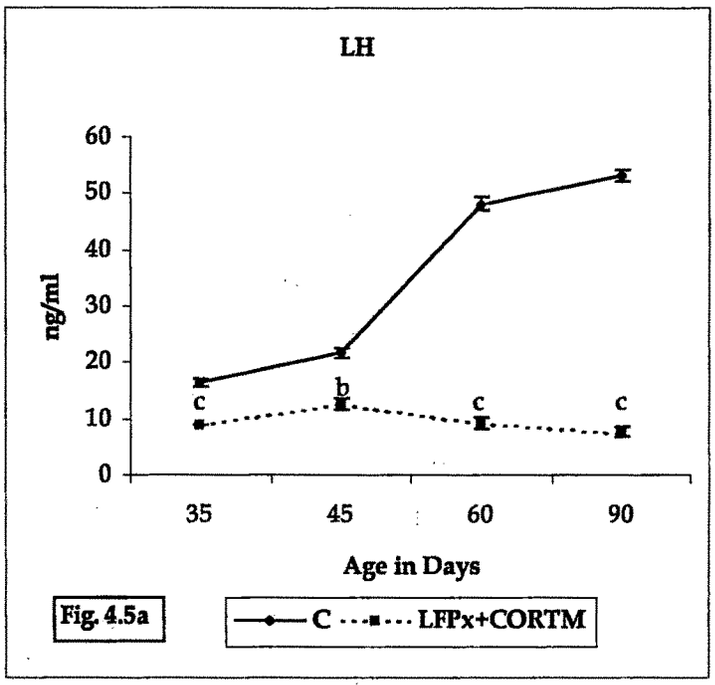


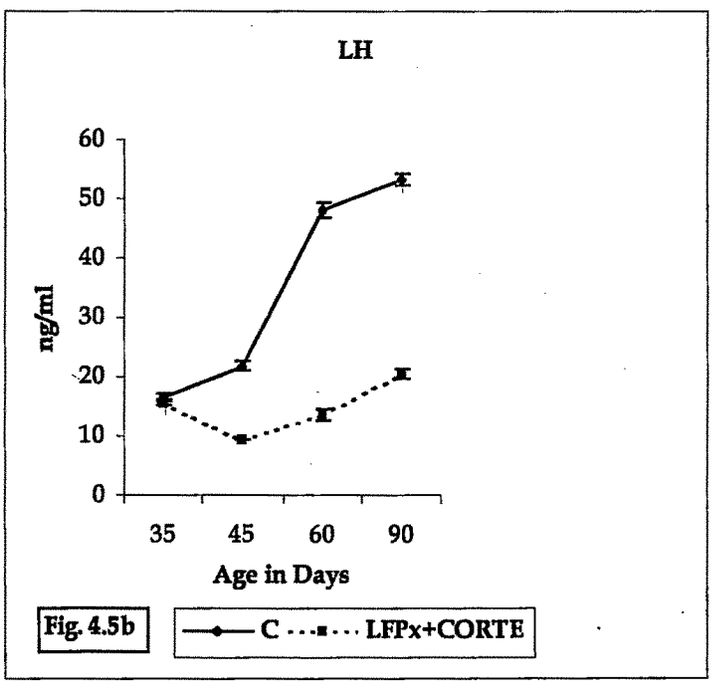
Fig. 4.4f

Figures 4.4e&f: Chronological alterations showing serum T4 (ng/ml) levels in control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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





Figures 4.5a&b: Chronological alterations showing serum LH (ng/ml) levels in control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

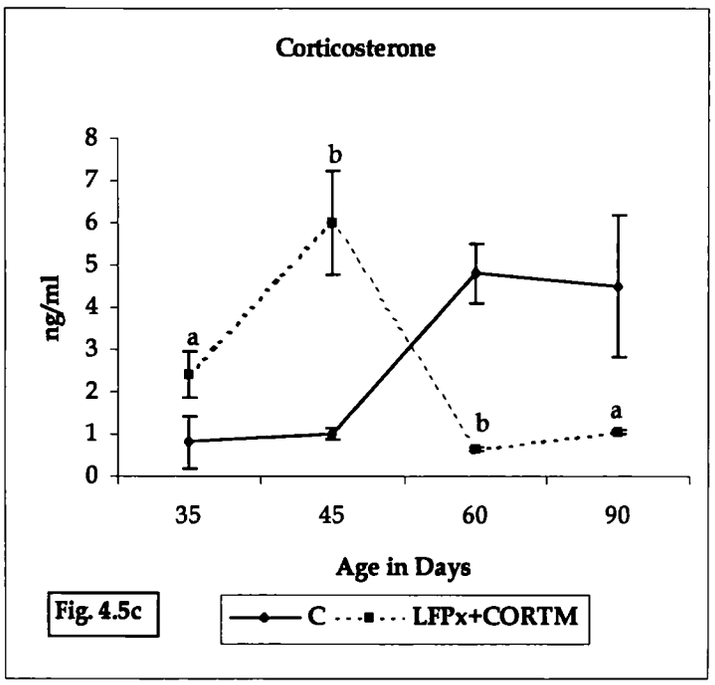


Fig. 4.5c

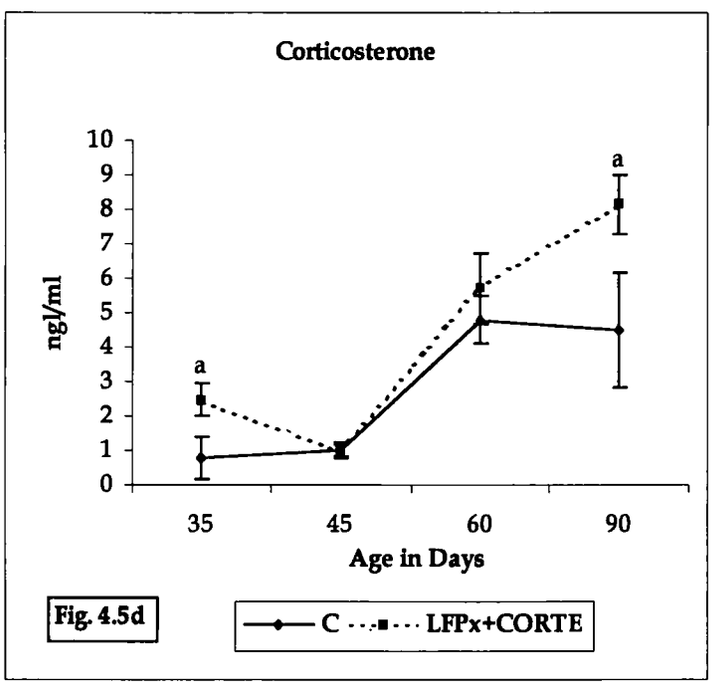


Fig. 4.5d

Figures 4.5c&d: Chronological alterations showing serum Corticosterone (ng/ml) levels in control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

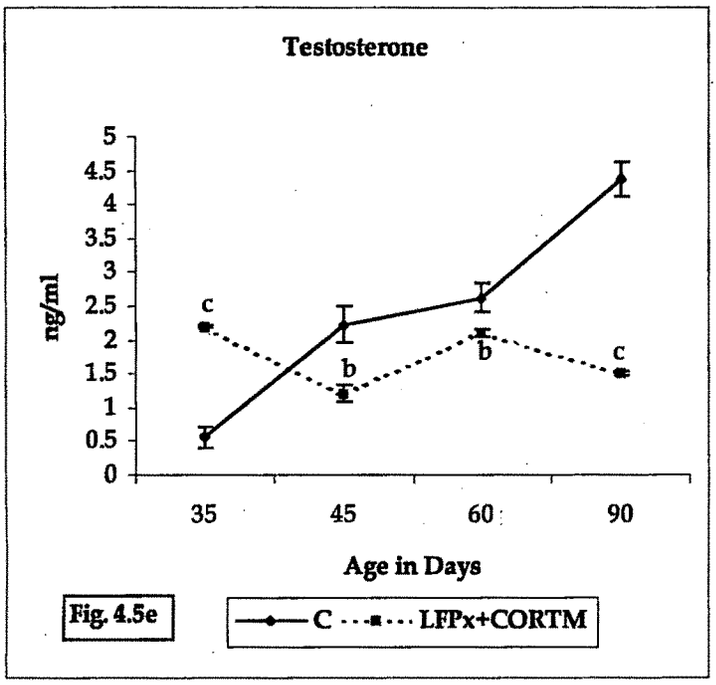


Fig. 4.5e

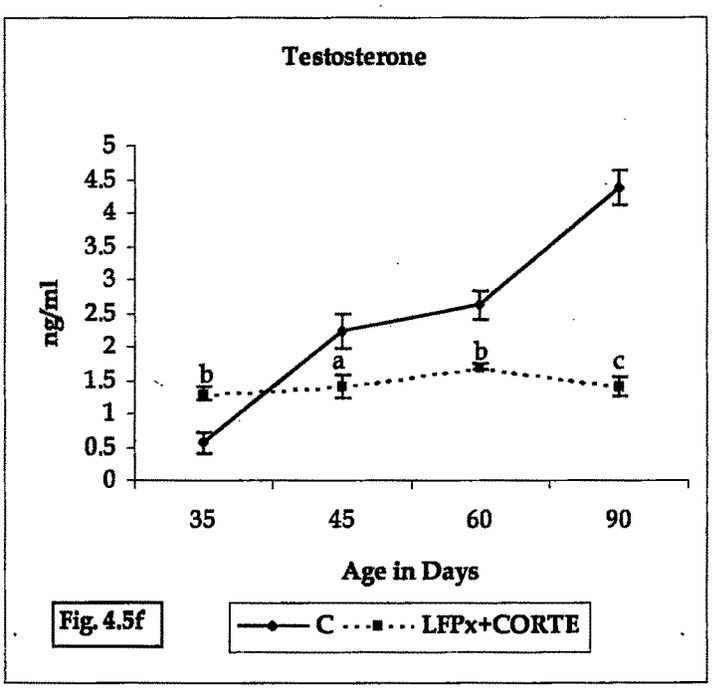


Fig. 4.5f

Figures 4.5e&f: Chronological alterations showing serum Testosterone (ng/ml) levels in control (C) and functionally pinealectomised corticosterone (morning and evening) treated ($LFPx+CORT_M$) and ($LFPx+CORT_E$) rats

Values expressed as Mean ± SEM of six animals;

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

DISCUSSION:

In a previous study, LFPx in rat neonates from day 0 to day 21 did not show any significant change in body weight and absolute testes weight at 90 days compared to controls, though there were higher growth rates initially (Chapter 1). But a previous study involving surgical pinealectomy (Px) showed increased adult body weight (Sharma and Ramachandran, unpublished). Apparently, effects of Px and LFPx differ slightly as in the former there is abolishment of pineal rhythm and in the latter hormone rhythm gets re-establish on return back to ambient condition. In the present study on simultaneous administration of corticosterone to neonates subjected to LFPx there is significant reduction in body weight and a significant increase in relative testis weight of LFPx+$CORT_E$ rats but not in LFPx+$CORT_M$ rats. This would suggest the potential favourable influence of LFPx+$CORT_E$ to increase testes weight. This favourable influence on testes weight is more due to CORT as a previous study on neonatal administration of CORT in the evening had shown a similar increase in testes weight (Bhavsar *et al.,* unpublished). Similar increase in testes weight at 45 days has also been recorded by Biagini and Pich (2002) in their study on neonatal corticosterone administration during SHRP. The above study also showed a similar time dependent effect of CORT on testes weight as both morning as well as evening CORT

administration increased adult testes weight but, in the present study, LFPx seems to nullify the morning CORT effect on testes weight. Early onset of spermatogenesis with appearance of sperms on 45 days seen in LFPx or CORT alone rats (Chapter 1; Bhavsar *et al.*, unpublished) could not be seen in the present study when LFPx and CORT treatment were combined. The early onset of spermatogenesis seen in LFPx or CORT in the above-cited studies was related with higher T4 and T3 levels in the pre-pubertal periods, which favour Sertoli cell differentiation (Maran and Aruldhas, 2002). The relatively low levels of T3 seen in the present experimental rats in the pre-pubertal period support the delayed spermatogenic progression caused due to slower Sertoli cell differentiation. A generalized hyposetting of the pituitary-thyroid axis in LFPx+CORT rats is indicated by the observed decrease in T3 and T4 levels and higher TSH level, more comparable with CORT treated rats. A combination of LFPx and CORT seems to have more detrimental effects on spermatogenesis as, not only the process per se progresses slowly but, there is also reduced population of sperms in the evening CORT treated animals which is further potentiated in $CORT_M$ rats. Apart from this inhibitory effect in spermatogenesis, the causes or reasons for which are unknown as of now, a time dependent effect with morning administration of CORT having a more pronounced effect is also inferable.

Comparison of the histometric enumeration of seminiferous tubules of control and LFPx+CORT rats reveals that apart from increased germinal epithelial thickness, there is also significant increase in tubular length, basement membrane area and total number of germ cells. This is in contrast to the changes observed in LFPx or CORT alone rats (Chapter 1; Bhavsar *et al.*, unpublished). Apparently, LFPx+CORT has effects on the above parameters of testis, distinct to those induced by LFPx or CORT alone. Though there is a generalized increase in the number of germ cells, it is also obvious that late spermatids and spermatozoa are poorly represented or are lost into the lumen by degenerative/apoptotic changes. This effect of reduced generation of sperms and or loss seems to be similar to that seen in CORT alone treated rats or even melatonin treated rats and, in both the above studies, it was correlated with CORT induced increase in apoptosis of germ cells (Bhavsar *et al.*, unpublished; Ramachandran *et al.*, 2004). The pre-pubertal increase in CORT has been related with increased number of advanced germ cells and like spermatids and sperms in LFPx+MT rats (Chapter 2) essentially due to altered adhesive property of Sertoli cells and advanced germ cells as seen earlier (Bhavsar *et al.*, unpublished). It is interesting that both neonatal MT and CORT administration in earlier studies had shown increased germ cell survival by preventing apoptosis (Bhavsar *et al.*, unpublished; Biagini and Pich,

2002; Ramachandran *et al.*, 2004). In the present study, simultaneous administration of CORT with LFPx does seem to manifest the supportive effect of neonatal CORT on survival of early germ cells but with an inhibitory effect on spermiogenesis coupled with loss of sperms due to some unknown effect of light exposure. A possible explanation may be sought in the subnormal levels of T3, testosterone and corticosterone from pubertal age onwards, as a long-term consequence of LFPx+CORT. It's likely that this hypo-hormonal *milieu* could be the definitive cause for the observed effect.

Based on the study on neonatal corticosterone administration during SHRP Biagini and Pich (2002) had inferred that SHRP could be a functional requirement for adequate development of rodent sexual potential. Weber et al. (2000) had shown that GR expression is down regulated in the tubular but not in Leydig cell compartment during SHRP. Increased immunoreacitvity in peritubular, Sertoli and spermatogenic cell nuclei of 45 day old rats treated with corticosterone in the neonatal SHRP period had been taken to indicate up regulation of GR binding in the proliferative compartment of spermatogenic cell (Biagini and Pich, 2002). This finding points to a role for CORT in altering some basic developmental mechanisms operative immediately after birth that could set in the long-term period the level of response to the

glucocorticoid in the basal zone of testicular tubules (Biagini and Pich, 2002). Apoptosis induced by glucocorticoids has been localized in the basal zone of tubule where increased GR immunoreactivity after CORT administration to rat pups has been recorded (Yazawa et a., 1999, 2000; Biagini and Pich, 2002).

Clearly, the above study suggests an apoptotic effect of glucocorticoid in germ cells as against of our observation of reduced apoptosis. This differential inference may be explained by the relatively different dosages and treatment schedules employed in the two studies as, Biagini and Pich, (2002) used a dosage of 10mg/kg body weight CORT for only a week from postnatal day 2 to 11 while, our studies have used only 1.5 to 3mg/kg body weight CORT for 21 days. Apparently, a low dose of CORT administered during the entire pre-weaning period has a favourable influence on reducing germ cell apoptosis in adult testis as against a much higher dose. This shows that CORT has dose dependent differential positive or negative influence on reproductive functions.

LFPx+CORT has a down regulating effect on thyroid, adrenal and gonadal neuroendocrine axes as seen by the lowered T3, T and CORT titres. Obviously, a combination of LFPx and CORT in the sensitive neonatal period has long lasting effects on the neuroendocrine axes. The prominent Leydig cell seen in the

present study is possibly a consequence of higher LH titres due to reduced negative feedback of testosterone and also indicates increased Leydig cell formation to compensate for the hyposensitivity.

Finally, it can be concluded from the present observations that simultaneous administration of CORT in LFPx neonates has favourable influence on immature germ cell survival but a detrimental effect on spermiogenesis, which is more potentiated by morning administration of CORT than in the evening and these effects on adult germ cell dynamics is quite distinct from those induced by LFPx or CORT administration alone.

SUMMARY:

The present study is aimed to assess the time dependent effect of corticosterone in light induced functionally pinealectomised rat neonates on adult testis functions and serum hormone profiles. The rat neonates were exposed to continuous light and simultaneously administered with corticosterone (1μg/animal/day from day 0 to 10 and 2 μg/animal/day from day 11 to day 21) in the evening and (2μg/animal/day from day 0 to 10 and 4 μg/animal/day from day 11 to day 21) in the morning. The body weight of LFPx+CORT animals was significantly less but there was no relative difference in absolute and relative testes weight in LFPx+$CORT_M$ or LFPx+$CORT_E$ rats compared to controls at 90 days. Histologically, testis of LFPx+$CORT_M$ or LFPx+$CORT_E$ showed no much difference. Fewer sperms and more number of early stages of germ cells with prominent Leydig cells were the key features compared to controls at 90 days. There was no significant difference in seminiferous tubular volume or total number of Sertoli cells of control and experimental rats, but the number of germ cells per meter length of tubules was significantly less in experimental rats at 90 days. Serum hormone titres of TSH and T4 were higher during pubertal age (45 days) whereas the T4 levels were significantly lesser in adults of LFPx+$CORT_M$ rats. The T3 levels showed significant reduction in LFPx+$CORT_M$ and LFPx+$CORT_E$ rats

compared to controls. Also serum LH and T showed reduced titres in both experimental groups at all ages except at 35 days than the age-matched controls. It can be concluded from the present observations that simultaneous administration of CORT in LFPx neonates has favourable influence on immature germ cell survival but deleterious effect on spermiogenesis, which is more potentiative in morning regimen.