CHAPTER - IV

Neonatal evening corticosterone excess potentiates detrimental effect of melatonin on spermatogenesis: I. Time dependent effect

Higher levels of glucocorticoids either due to disease of the adrenal or stress, and also experimental exposure to exogenous glucocorticoids, have been reported to affect male gonadal functions, including steroidogenesis (Kumar and Rao, 1976; Blair and Light, 1984; Collu *et al.,* 1984; Orr *et al.,* 1994; Maric *et al.,* 1996; Chatterton *et al.,* 1997; Harriba, 1997). Corticosterone during foetal development has been shown to affect postnatal reproductive development in both the sexes (Harvey and Chevins, 1987; Politch and Herrenkohl, 1994; Smith and Waddell, 2000) marked by delayed puberty onset. In a previous study we had examined the effect of glucocorticoid excess in the immediate postnatal period (preweanling) and found that small doses of corticosterone within the physiological range are favourable for early testes maturation when given in the evening (Chapters 2 and 3).

The pineal hormone melatonin, has been shown to retard reproductive development in both sexes when administered in the immature stages (Wurtman *et al.,* 1963; Motta *et al.,* 1967; Debeljuk, 1969; Kinson and

Robinson, 1970; Kinson and Peat, 1971; Lang *et al.,* 1983). A time specific dose dependent inhibitory action of melatonin on sexual maturation in the rat has also been shown when given daily in the afternoon from 20-40 days (Lang *et al.,* 1983) but with no effect when given either from 5-20 days or in the adulthood from 70-90 days (Lang *et al.,* 1983). Though a delay in sexual maturation was observed with melatonin treatment during 20-40 days of age, there was nevertheless no inhibition as development and functioning of reproductive system were found to be normal (Lang *et al.,* 1984). An earlier study from this laboratory had reported decreased body and testes weight due to melatonin treatment between 10 and 25 days of age (Patel and Ramachandran, 1992). The above study also showed that the retardatory influence can be manifested by both morning as well as evening administration, though more pronouncedly by evening administration. Since most of the above studies involved investigating the influence of melatonin during short periods of postnatal exposure, our previous study evaluated the long-term influence of exogenous melatonin during the first 21 days of neonatal life, on adult testes size, structure and function (Chapter 1). The above study showed increased germ cell number but decreased survival of advanced germ cells as long-term effects of evening melatonin. Moreover, the previous studies involving either glucocorticoid or melatonin excess have both been shown to affect adrenal, thyroid and reproductive endocrine axes.

In the light of above observations, it was thought feasible to evaluate the influence of neonatal excess of both glucocorticoid and melatonin on postnatal maturation of testes and adult functions. To this end, in the present study the influence of evening melatonin in neonates rendered hypercorticalic by evening administration (low dose) of corticosterone from day 0 to day 21, has been evaluated in terms of adult testes weight, spermatogenic and steroidogenic functions and hormonal profiles in the post weanling period till adulthood.

MATERIAL AND METHODS:

Animals and Maintenance:

As in chapter one.

Preparation of Melatonin and Corticosterone:

As in chapters one and two.

Experimental Protocol:

The experimental set-up was divided into two major groups of study, both consisting of subgroups as mentioned below.

Group I (control) **(C)**:

Newborn rat pups maintained till 90 days served as controls. This consisted of 2 subgroups (as follows) of 30 animals each:

i. Control rats

ii. Injected *i.p.* with vehicle (0.9% saline) in evening (1600 hrs)

Group II (Melatonin + Corticosterone):

This experimental group consisted of 30 rats injected with a combination of melatonin and corticosterone in doses as mentioned previously (Chapters 1 and 2).

(i) Melatonin and CORT (Low dose) injections (*i.p.*) were given in the evening (1600 hrs) **(CE+MT).**

Parameters and Methods of Evaluation:

As in chapter one.

Histology and Histometry:

As in chapter one.

Hormone Assays:

As in chapter one.

Statistical Analysis:

As in chapter one.

RESULTS:

Since no significant difference was observed between vehicle and non-vehicle controls, the data represented is of vehicle control **(C)** only.

Postnatal Growth:

The body weight of CE+MT rats was greater than the controls though, it was marginally high. Nevertheless, during the treatment period and post-treatment period upto 60 days, the weights of these animals were significantly higher as denoted by the higher growth rates (Tables 1 & 2; Figures 1a, 1b, 3a & 3b). Whereas the peak growth rate was seen between 45-60 days in CE+MT animals as in the controls, the growth rate during 60-90 days was significantly lesser. The body weight of CE+MT at 90 days was significantly lower than melatonin treated animals and significantly higher than the corticosterone treated animals (CE). The absolute weight of testes at 90 days was significantly lesser than that of controls and identical to that seen in CE animals. The weight of testes was higher in experimental animals upto 45 days, which is reflected in the higher growth rates. Whereas the testes of CE animals showed maximum growth rate during 35-45 days, the same in CE+MT was between 45-60 days, the latter being similar to controls. The relative weight of testes, except at 15 and 35 days (treatment and immediate post-treatment period), which was higher than the control, was lesser than the control at all other stages (Table 1; Figure 2). The relative

The data of:

- Body weight and Testes weight
- Relative weight
- Body growth rate and Testes growth rate
- Corticosterone level
- LH and T levels
- TSH level
- T_4 and T_3 levels

of treatments MT and CE has been recalled from chapters 1 and 2 respectively.

Table 1: Chronological alterations in body weight (g) and absolute (g) and relative weight (g/100 g) of testes in Control and Corticosterone + Melatonin treated rats

Treatment	Body Weight					Testes Weight					Relative Testes Weight				
	Age in days					Age in days					Age in days				
	15	35	45	60	90	15	35	45	60	90	15	35	45	60	90
C	31 00 ±1.654	90.34 ±2.03	130.2 ±5.78	209.89 ±9.03	340.7 ±6.782	0.208 ±0.019	0.808 ±0.059	1.389 ±0.050	2.698 ±0.086	3.354 ±0.1	0.67 ±0.016	0.894 ±0.021	1.066 ±0.033	1.285 ±0.035	0.984 ±0.029
CE + MT	35.5[a] ±1 843	113.7[c] ±1.926	157.0[a] ±2.937	266.6[b] ±5.649	365.4 ±6.258	0.252[c] ±0.024	1.027[b] ±0.021	1.503[c] ±0.04	2.567[c] ±0.076	3.230[c] ±0.066	0.79[c] ±0.019	0.943 ±0.021	0.948[a] ±0.024	0.950[c] ±0.03	0.888[a] ±0.017
* C	37 33 ±1.99	85.573 ±1.902	117.16 ±2 307	193.66 ±5.493	322.4 4.078	0.218 ±0 017	0.803 ±0.061	1.28 ±0.09	2.28 ±0.08	3.067 ±0.102	0 755 ±0.002	0 944 ±0.025	1.09 ±0.040	1 178 ±0.073	0.933 ±0.011
MT	31 45[a] ±1 477	110.3[c] ±2.490	152.3[c] ±2.490	233 7[c] ±3.242	398.7[c] ±9.898	0.231[a] ±0.061	0.938 ±0.073	1.779[c] 0.053	2.714[a] ±0.102	3.375[c] ±0.078	0.677 ±0.081	0.854 ±0.084	1.167 ±0.042	1.16 ±0.063	0.847 ±0.043
* C	32.64 ±1.754	93.66 ±2.958	122.5 ±5.155	200.6 ±2.172	349.10 ±7.902	0.321 ±0.020	0.938 ±0.067	1.404 ±0.046	2.478 ±0.095	3.331 ±0.124	0.983 ±0.071	1.002 ±0.063	1.146 ±0.047	1.235 ±0.051	0.954 ±0.044
CE	24 87[a] ±2.720	109.1[a] ±4.053	160.9[c] ±3.439	240.2[c] ±4.945	332.67 ±10.58	0.218[b] ±0.024	0.984 ±0.047	1.805[c] ±0.063	2.509 ±0.085	3.243 ±0.067	0.875 ±0.048	0.904 ±0.044	1.128 ±0.057	0.921[c] ±0.089	0.967 ±0.020

C – Control, **CE +MT** - Low Dose Corticosterone evening injection + Evening Melatonin injection, **CE** - Low Dose Corticosterone evening injection **MT** - Evening Melatonin injection

Values expressed as Mean ± SEM of six animals. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

* Values recalled from Chapters 1 and 2

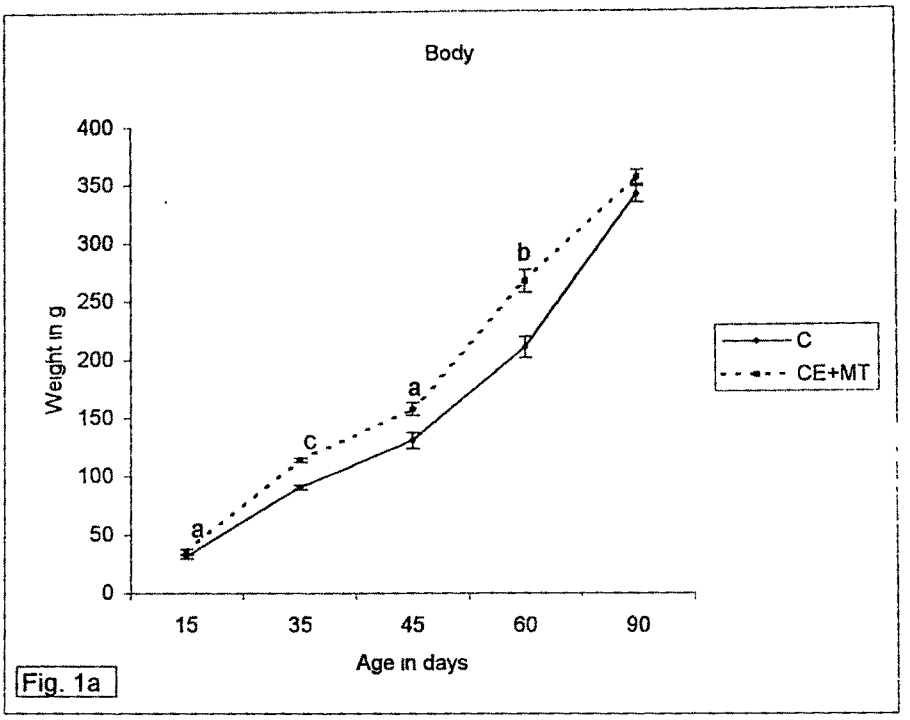


Fig. 1a

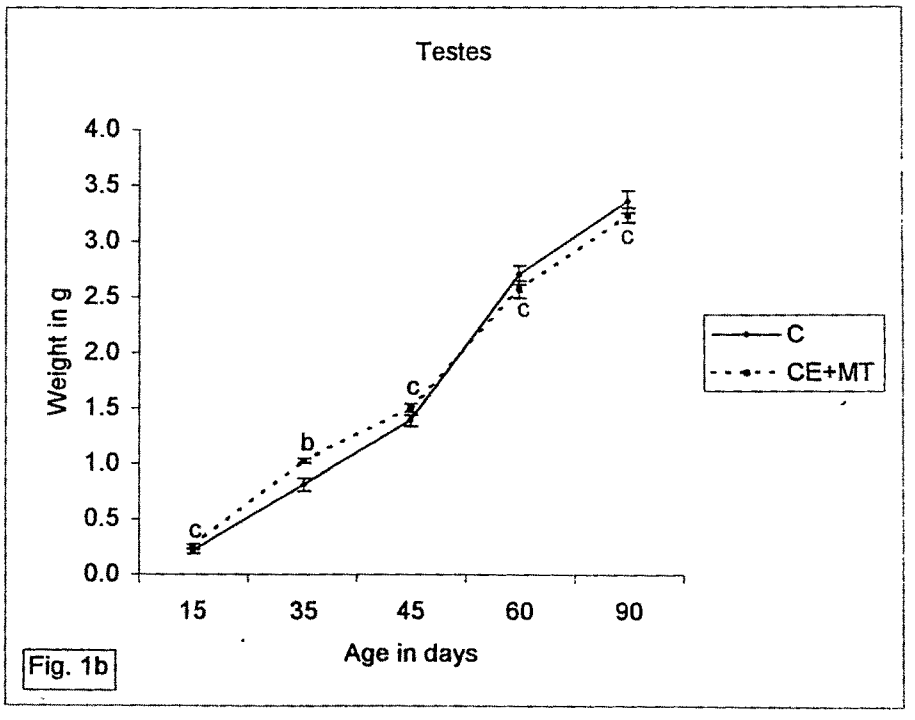
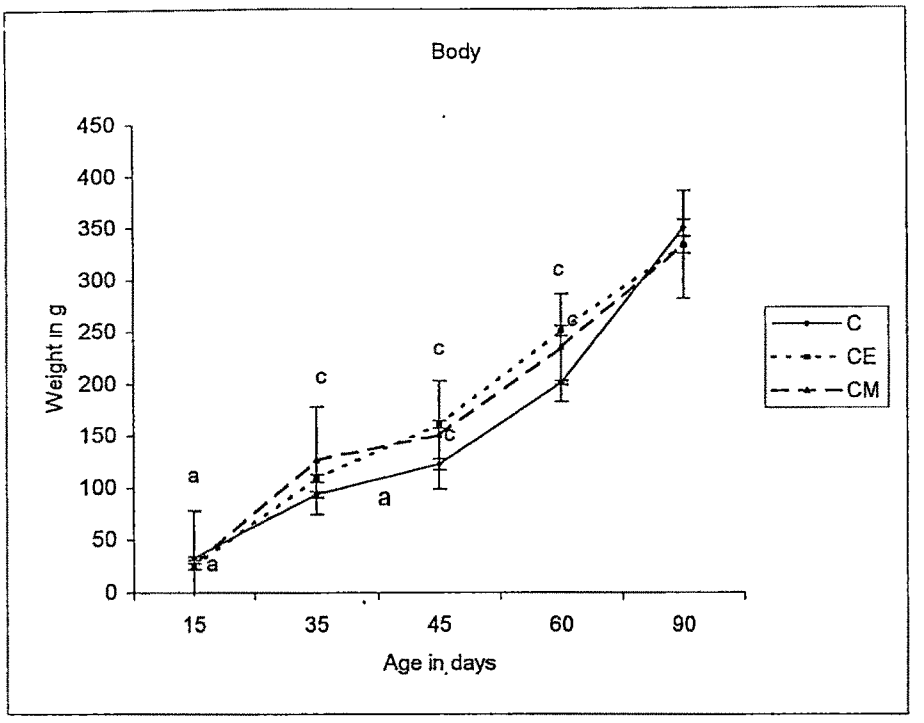
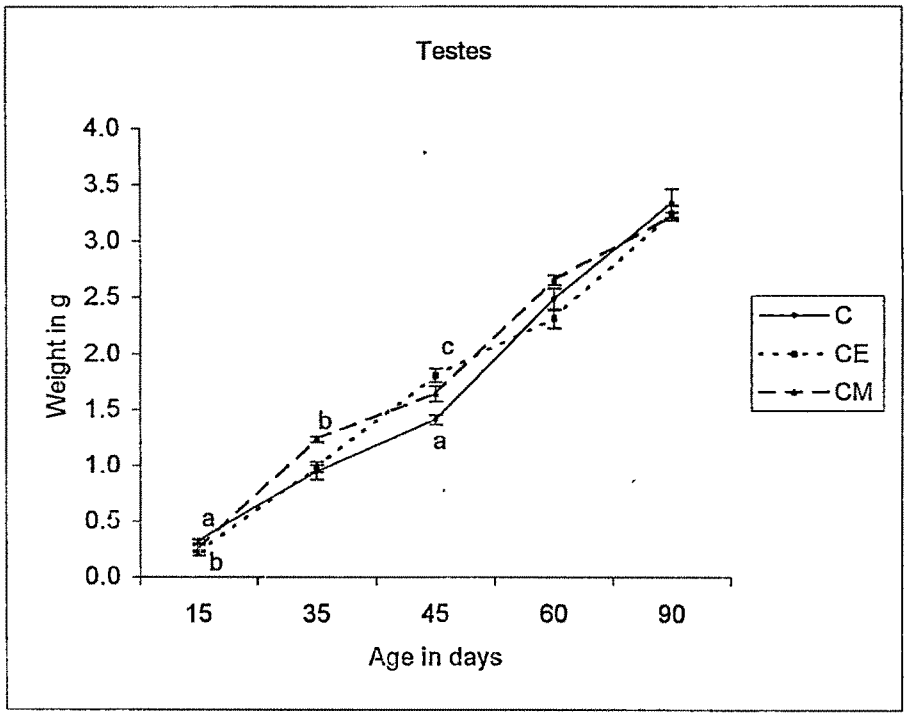


Fig. 1b

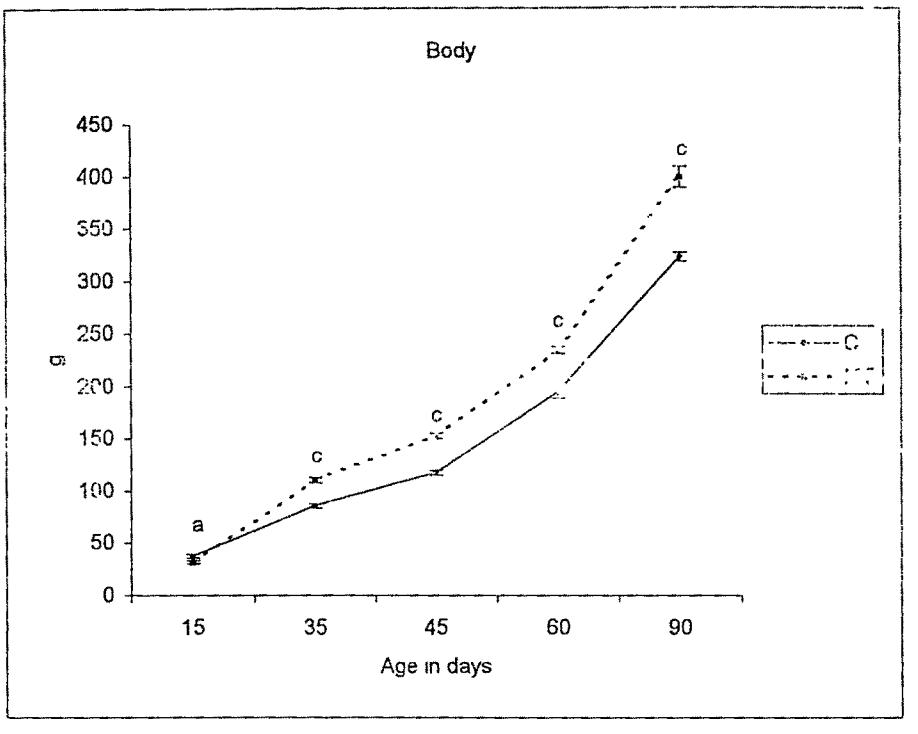
Fig. 1a and1b Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Corticosterone + Melatonin treated rats
C - Control, **CE+MT** - Low Dose Corticosterone evenin injection+Evening Melatonin injection, Values expressed as Mean ± SEM
of six animals [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0\ 0005$

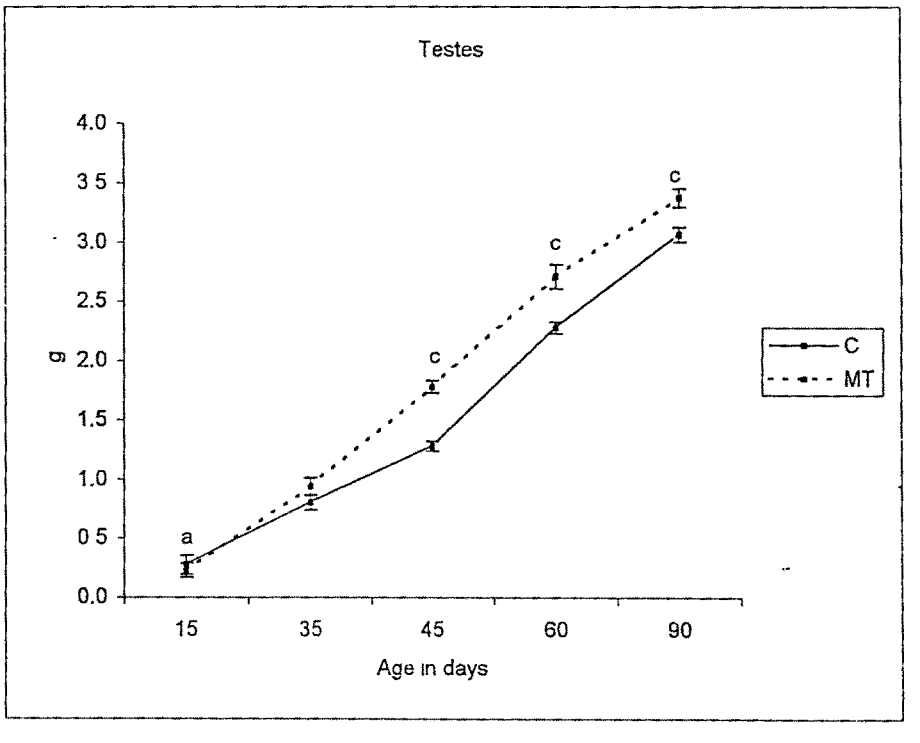




Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Corticosterone treated rats

C - Control, **CE** - Low Dose evening Corticosterone injection, **CM** - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of six animals, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$





Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Melatonin treated rats

C – Control, MT – Melatonin treated

Values expressed as Mean ± SEM of six animals. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

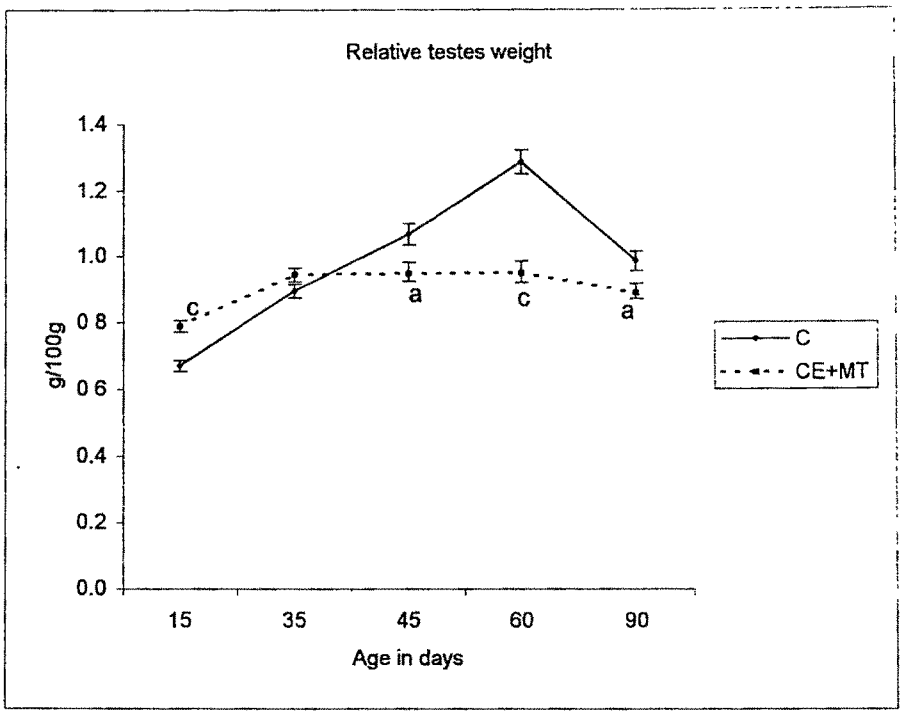
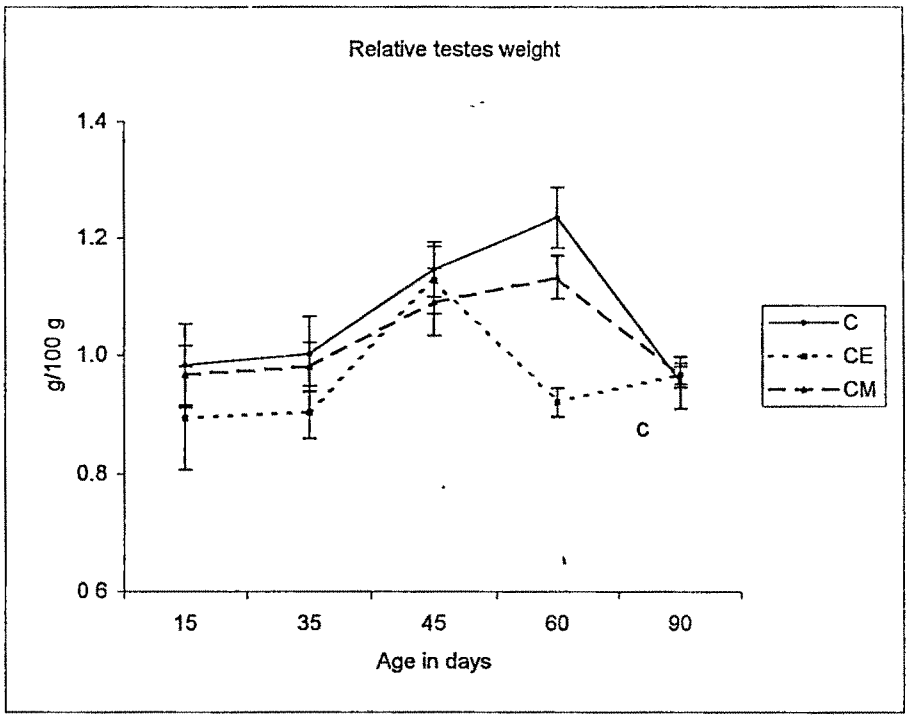
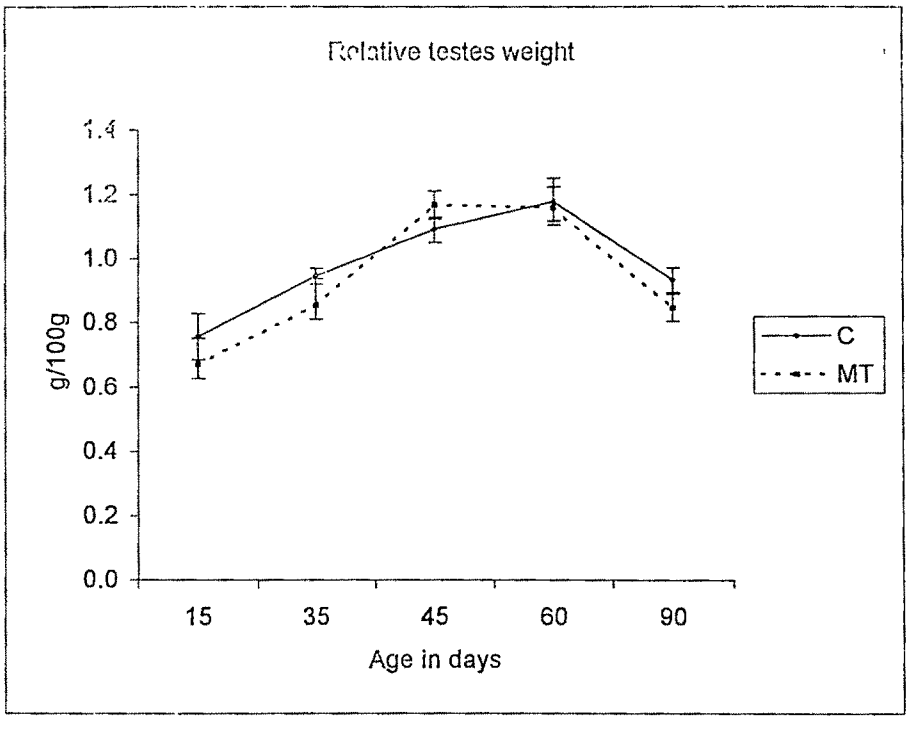


Fig. 2 Chronological alterations in Relative weight (g/100 g) of testes in Control and Corticosterone + Melatonin treated rats
C - Control, **CE+MT** - Low Dose Corticosterone evenin injection+Evening Melatonin injection, Values expressed as Mean ± SEM of six animals [a] $p< 0.05$, [b] $p< 0.005$, [c] $p< 0.0005$



Chronological alterations in relative weight (g/100 g) of testes in Control and Corticosterone treated rats

C - Control, CE - Low Dose evening Corticosterone injection, CM - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of six animals, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$



Relative testes weight (g/100g) in Control and Melatonin treated rats

C – Control, MT – Melatonin treated

Values expressed as Mean ± SEM of six animals. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

Table 2: Per day Body and Testes Growth Rate (g/day) in Control and Corticosterone + Melatonin treated rats.

Treatment	Per Day Body Growth Rate					Per Day Testes Growth Rate				
	Age in Days					Age in Days				
	0-15	15-35	35-45	45-60	60-90	0-15	15-35	35-45	45-60	60-90
C	1.695	2.967	3.986	5.313	4.360	0.014	0.030	0.189	0.044	0 022
CE + MT	1.923	3 908	4.334	7.307	2.967	0.017	0.039	0.048	0.071	0.022
C *	2.117	2 409	3.165	5.100	4.294	0 19	0.0261	0.148	0.052	0.102
MT	1.663	3.942	4.200	5.422	5.500	0.014	0.035	0.084	0.062	0.022
C *	1.804	3 048	2.890	5.207	4.950	0.021	0.031	0.045	0.072	0.028
CE	1.234	4.215	5.167	5.978	2.739	0.014	0.038	0.082	0.046	0 024

C – Control, **CE + MT** - Low Dose Corticosterone evening injection + Evening Melatonin injection, **CE** - Low Dose Corticosterone evening injection, **MT** - Evening Melatonin injection

* Values recalled from Chapters 1 and 2

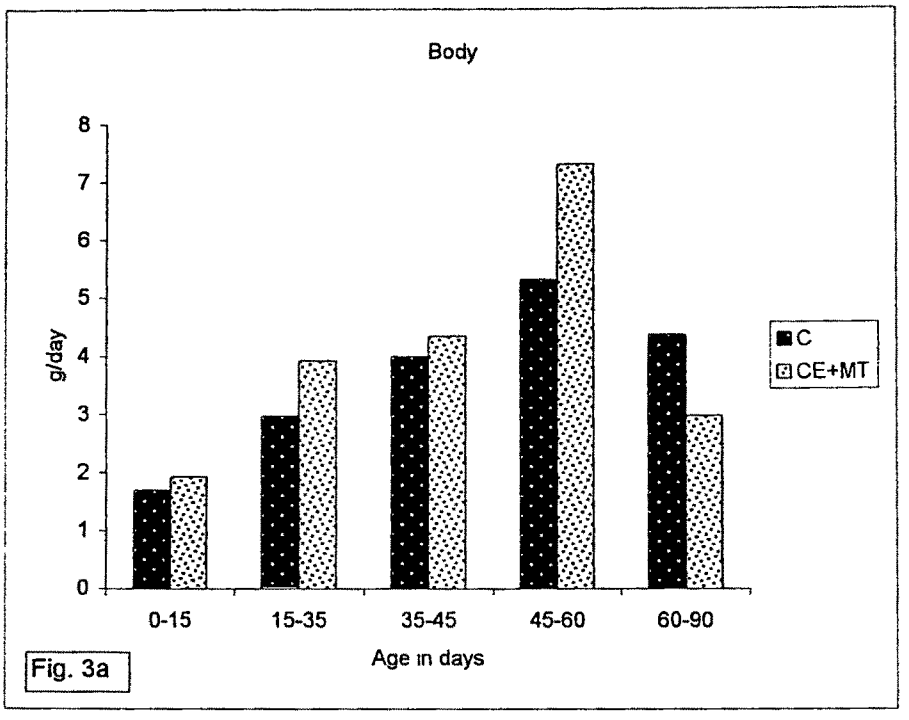


Fig. 3a

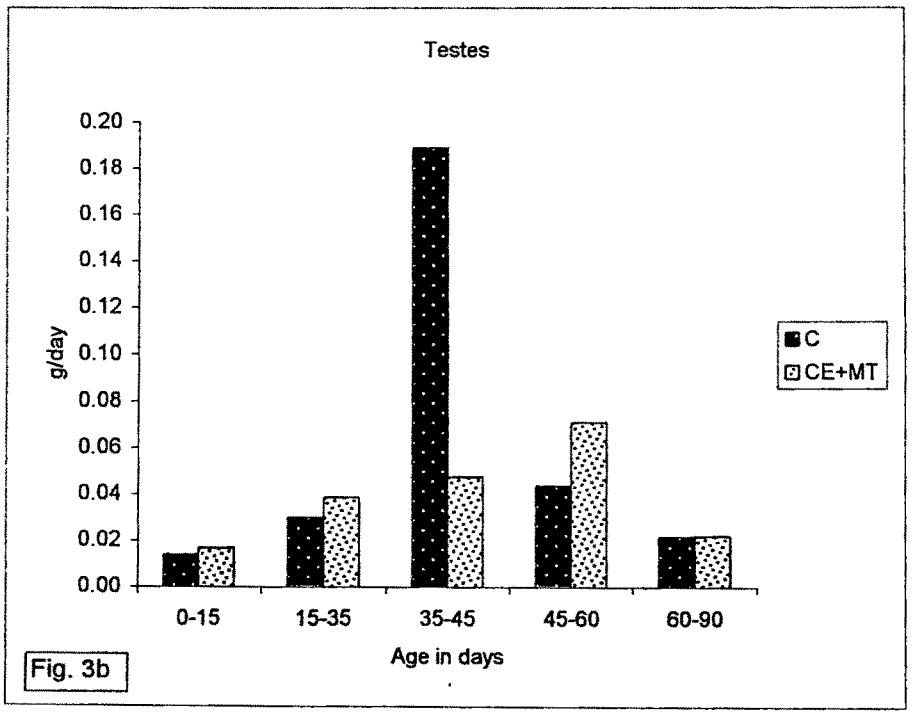
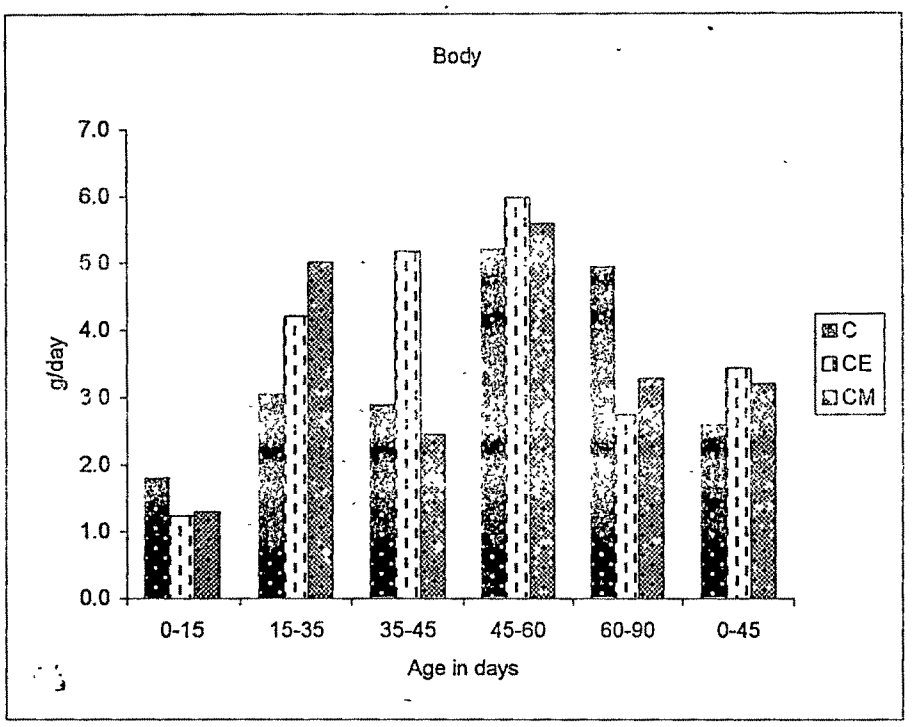
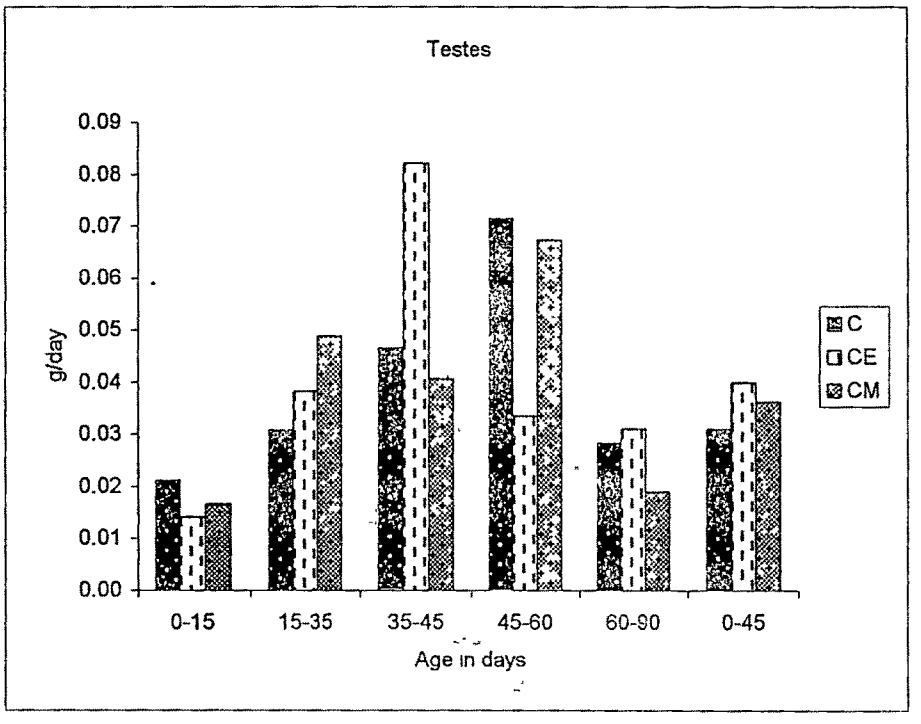


Fig. 3b

Fig. 3a and 3b Per day Body and Testes growth rate (g/day) in Control Coritcosterone + Melatonin treated rats

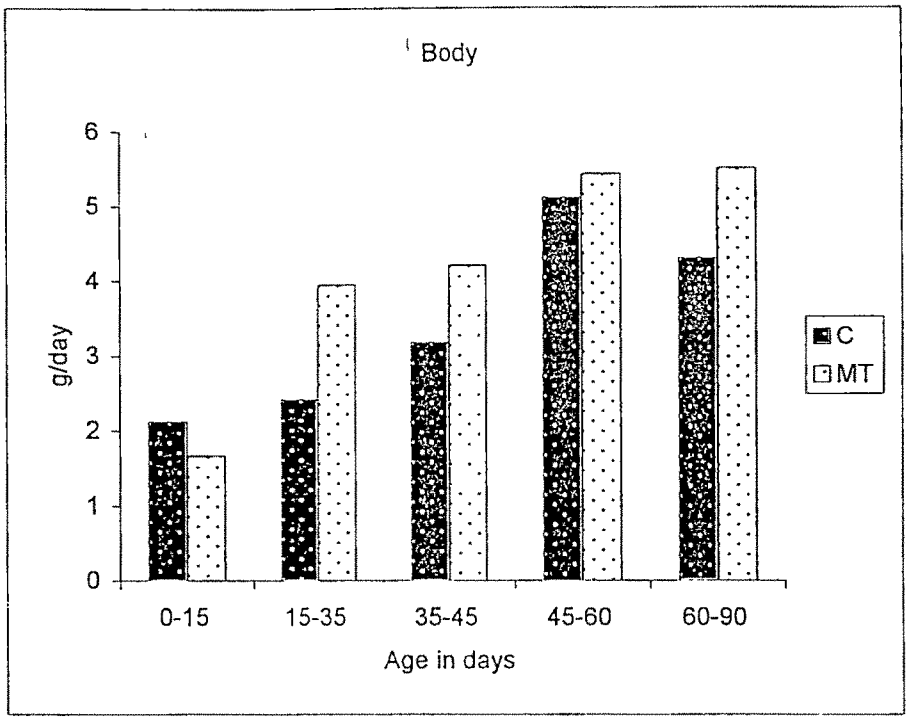
C - Control, **CE+MT** - Low Dose Corticosterone evenin injection+Evening Melatonin injection

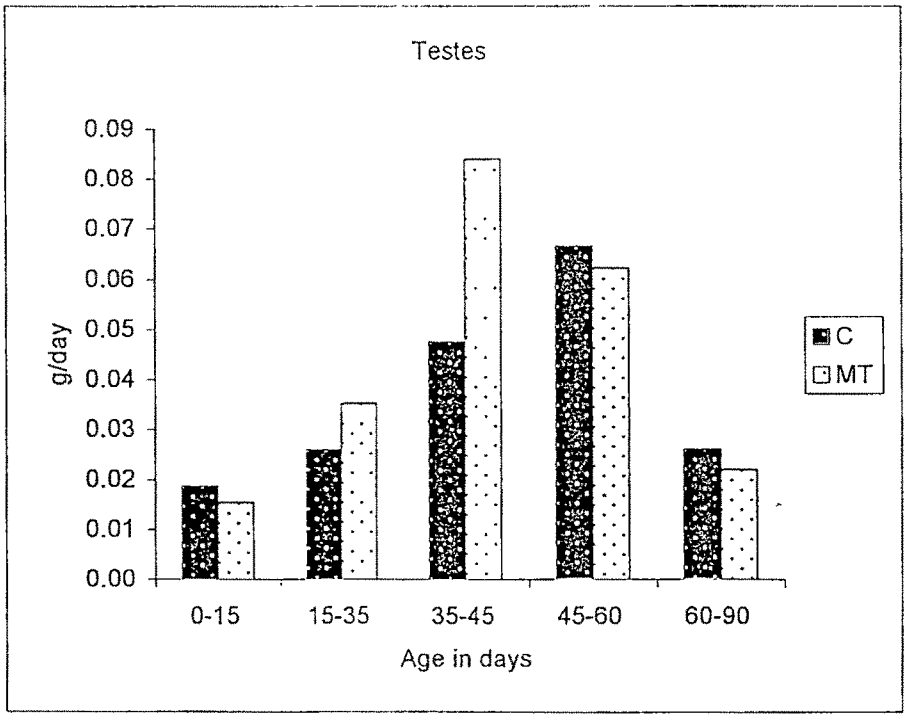




Per day Body and Testes Growth rate (g/day) in Control and Corticosterone treated rats

C - Control, **CE** - Low Dose evening Corticosterone injection, **CM** - Low Dose morning Corticosterone injection





Per day Body and Testes growht rate (g/day) in Control and Melatonin treated rats

weight of CE+MT testes at 90 days was greater than that of melatonin treated animals but significantly less than that of CE animals.

Histology and Histometry:

The testis and tubular volumes were greater in the experimental animals. The testis section of CE+MT animals at 35 days showed reduced tubular diameter with high degree of germ cell degeneration and loss. The tubular length and total basement area were significantly increased relative to melatonin treated animals but not in relation to controls or, corticosterone treated. Seventy-two percentage growth in tubular length had occurred by 35 days. Though there was a gradual increase in tubular diameter between 35 and 60 days, the trend of germ cell degeneration persisted. Completion of spermatogenesis and appearance of sperms were seen only at 60 days as in the controls. Though there was more compactly arranged germ cells, there was significant loss of germ cells as seen by empty spaces within the germinal epithelium as well as premature sloughing off of advanced stages of germ cells like spermatids and spermatozoa. The tubular diameter of CE+MT animals at 90 days was greater than that of controls and the germinal epithelium thickness showed similar thickness. The sperm mass in the tubules was significantly less due to premature detachment and dissociation. Total Sertoli cell number was not significantly different compared to controls or corticosterone treated animals, but it was significantly more compared to

Table 3: Histometric enumeration of seminiferous tubules of Control and Corticosterone + Melatonin 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	204.045 ±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
CE+MT	1.769 ±0.900	0.0285 ±0.0001	0.0074 ±0.0002	1.694 ±0.180	2321.59 ±35.600	222.32^b ±3.600	32.502 ±2.010	350.0^c ±6.200	280.0 ±2.900	15.17^c ±0.390	12.06 ±0.369	19.90^c ±0.250
* MT	1.541 ±0.070	0.033^c ±0.001	0.0098 ±0.002	1.448 ±0.060	1725.2^c ±45.30	177.20^c ±4.690	24.15^b ±1.600	340.00^b ±5.600	279.45 ±2.800	19.70^c ±0.350	$16\ 20^c$ ±0.210	19.00^c ±0.200
* CE	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, **CE+MT** –Low Dose Corticosterone evening injection + Evening Melatonin injection
Values expressed as Mean ± SEM of minimum fifteen observations. $^a p < 0.05$, $^b p < 0.005$, $^c p < 0.0005$

* Values recalled from Chapters 1 and 2

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.

melatonin treated animals. The actual germ cell number was similar to controls and MT treated rats but significantly less than those of corticosterone treated animals. The actual germ cell number is significantly less than the calculated theoretical total resulting in 20% germ cell loss (Table 3) (Plates I, II, VIIa & VIIb).

Serum Hormone Profile:

Corticosterone:

Control animals showed a gradual slow increase in serum corticosterone level from 15-45 days. Between 45-60 days there was significant increase by five times and the level remained steady thereafter. In the CE+MT animals, the hormone level during the treatment and immediate post-treatment periods (15 and 35 days) was significantly elevated by three times and five times respectively compared to corresponding control levels. Between 35-45 and 45-60 days, the level dropped to half the level at 35 days. The corticosterone titre further decreased significantly by 90 days to reach the lowest level (Table 4; Figure 4).

TSH, T_4 and T_3:

The levels of TSH, T_3 and T_4 increased continuously from a lower level at 15 days to the highest adult levels by 60 days (TSH and T_4) or 90 days (T_3). The levels of all the three hormones in CE+MT rats were significantly higher during the treatment period (15 days) as well as the immediate post-

Table 4: Serum Corticosterone, LH and T levels (ng/ml) in Control and Corticosterone + Melatonin treated rats.

Treatment	Corticosterone					LH					T				
	Age in days					Age in days					Age in days				
	15	35	45	60	90	15	35	45	60	90	15	35	45	60	90
C	5.825 ±0.085	8.000 ±0.618	10.150 ±0.155	48.300 ±0.705	45.00 ±1.699	9.390 ±0.132	16.450 ±0.634	21.7 ±0.854	48.125 ±1.235	53.250 ±1.031	0.235 ±0.019	0.550 ±0.166	2.235 ±0.278	2.625 ±0.217	4.375 ±0.265
CE + MT	20.8 ^{c} ±1 62	43.0 ^{c} ±3 26	21.0 ^{b} ±2.01	20.3 ^{c} ±1.69	10.0 ^{c} ±0.026	13.43 ^{a} ±1.24	28.54 ±3.89	40.17 ±2.46	74.34 ±3.82	56 5 ±3.02	0.40 ^{c} ±0.012	2.0 ^{c} ±0.019	2.3 ±0.016	5.2 ^{c} ±0 042	2.7 ^{a} ±0.027
* MT	12.40 ^{c} ±1.106	24.70 ^{c} ±2.075	13.40 ^{a} ±1.028	44.01 ±4 732	43.01 ^{c} ±4.856	8.761 ±1.009	12.17 ^{b} ±1.024	16.39 ^{b} ±1.008	17.77 ^{c} ±1.025	20.99 ^{c} ±2.025	0.221 ±0.016	1.501 ^{c} ±0.048	1.503 ^{a} ±0.054	1.300 ^{c} ±0.058	2.002 ^{b} ±0.088
* CE	15 60 ^{c} ±0.653	13.10 ^{c} ±0.208	13.25 ^{c} ±0.132	10.05 ^{c} ±0.522	9.100 ^{c} ±0.227	11.77 ^{c} ±0.094	20.57 ^{b} ±0.776	10.65 ^{c} ±1.028	14.00 ^{c} ±1.135	26.87 ^{c} ±0.527	0.333 ^{b} ±0.013	1.600 ^{b} ±0.122	1.550 ^{a} ±0.155	1.100 ^{c} ±0.041	1.000 ^{b} ±0.082

C – Control, **CE + MT** - Low Dose Corticosterone evening injection + Evening Melatonin injection, **CE** - Low Dose Corticosterone evening injection **MT** - Evening Melatonin injection

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

* Values recalled from Chapters 1 and 2

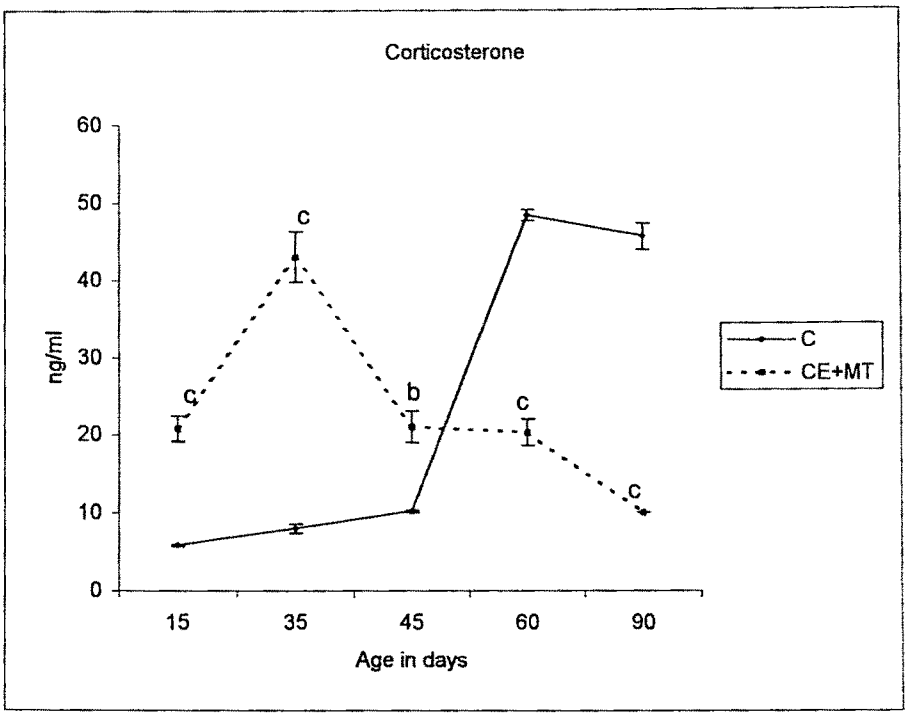
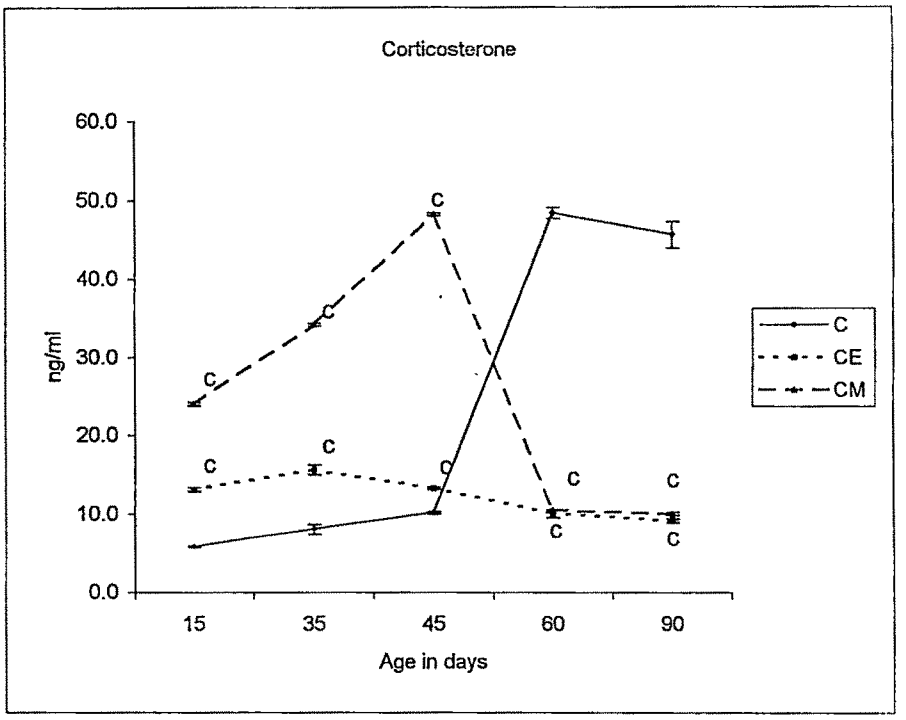


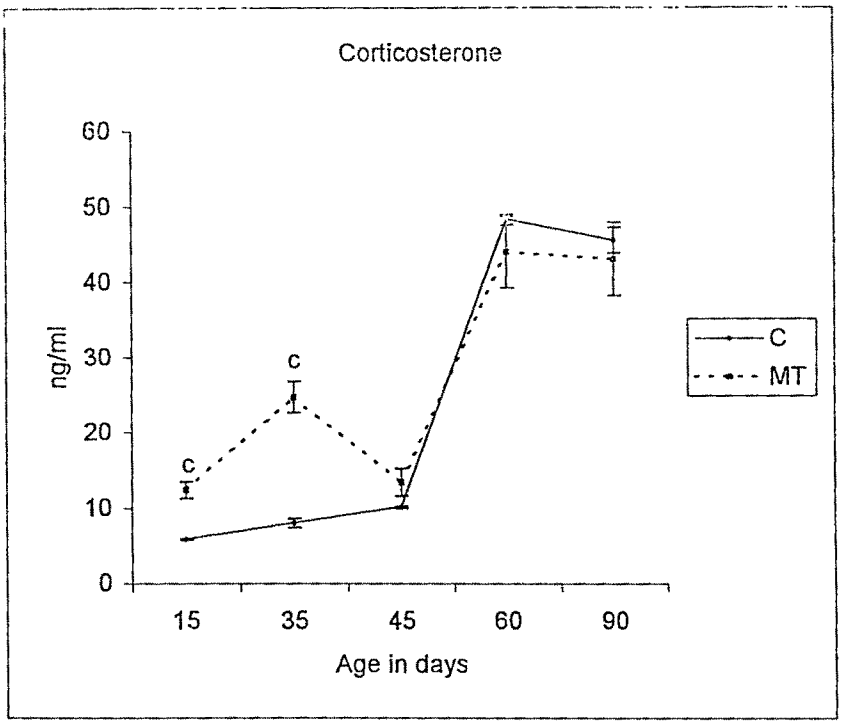
Fig. 4: Serum Corticosterone (ng/ml) level in Control and Corticosterone + Melatonin treated rats

C - Control, **CE+MT** - Low Dose Corticosterone evenin injection+Evening Melatonin injection, Values expressed as Mean ± SEM of four samples [a] $p< 0.05$, [b] $p< 0.005$, [c] $p< 0.0005$



Serum Corticosterone level (ng/ml) in Control and Corticosterone treated rats

C - Control, CE - Low Dose evening Corticosterone injection, CM - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of four samples, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$



Serum Corticosterone level (ng/ml) in Control and Melatonin treated rats

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

a $p < 0.05$, b $p < 0.005$, c $p < 0.0005$

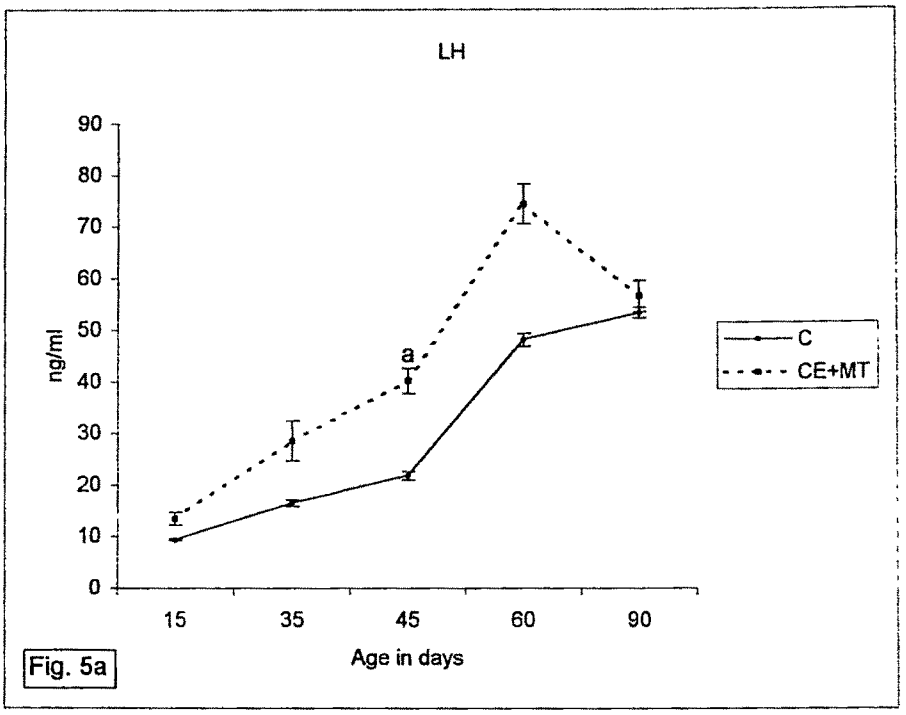


Fig. 5a

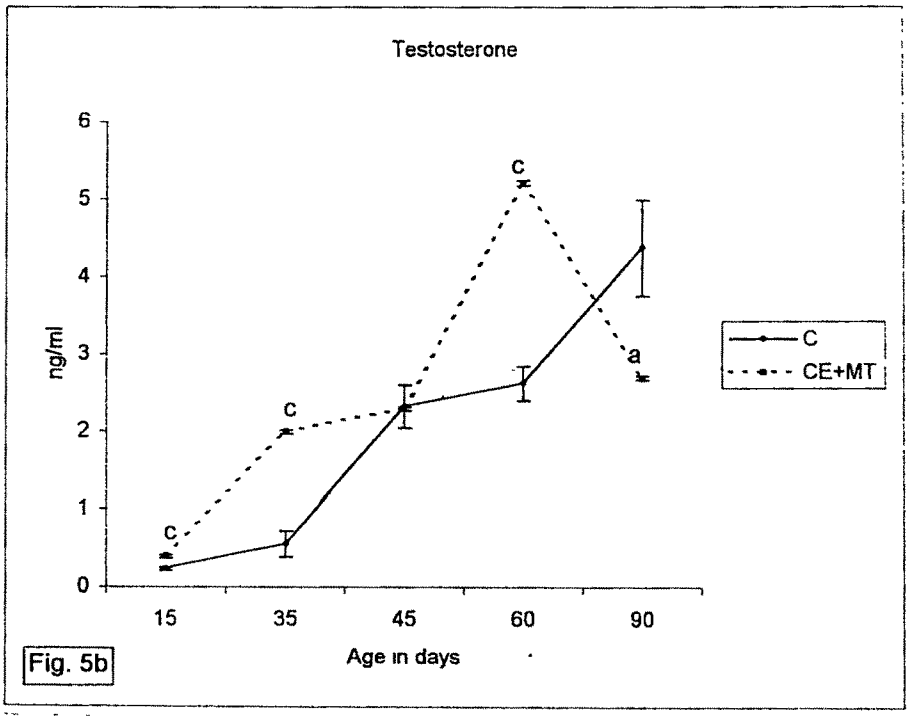
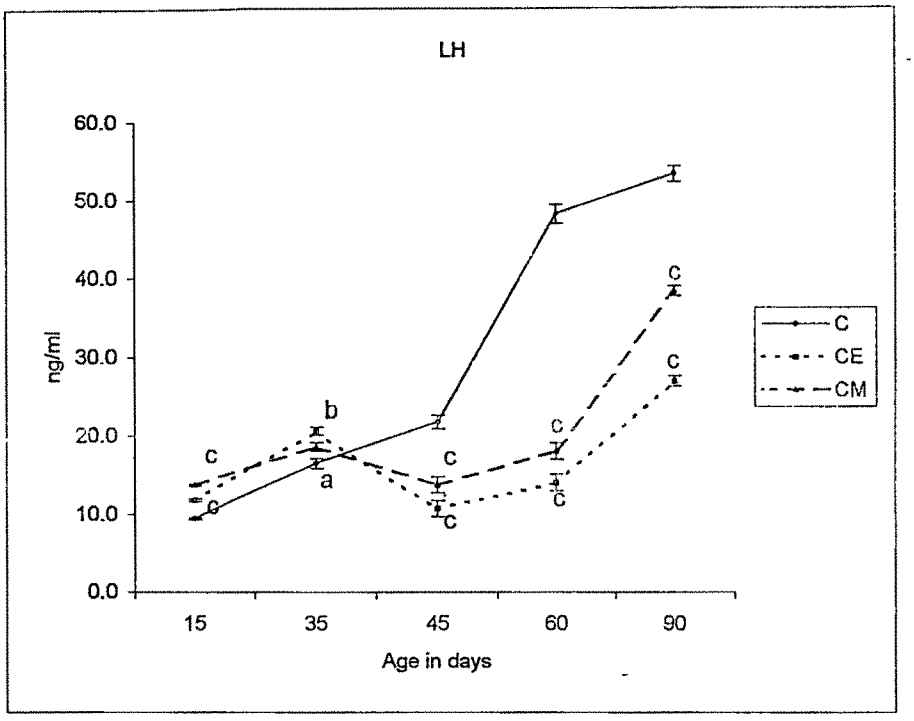
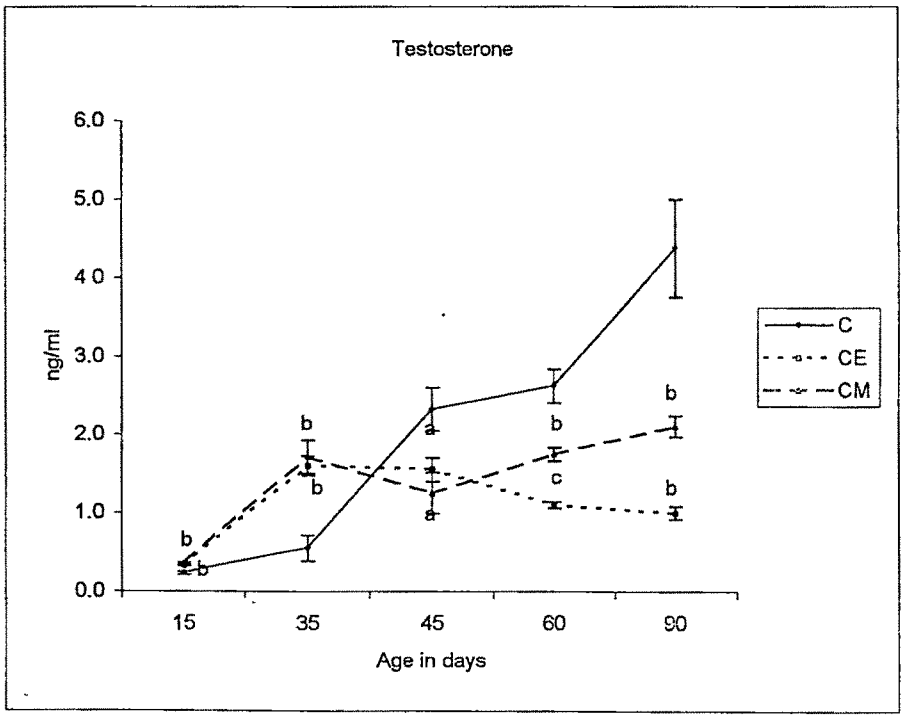


Fig. 5b

Fig. 5a and 5b: Serum LH and T (ng/ml) levels in Control and Corticosterone + Melatonin treated rats

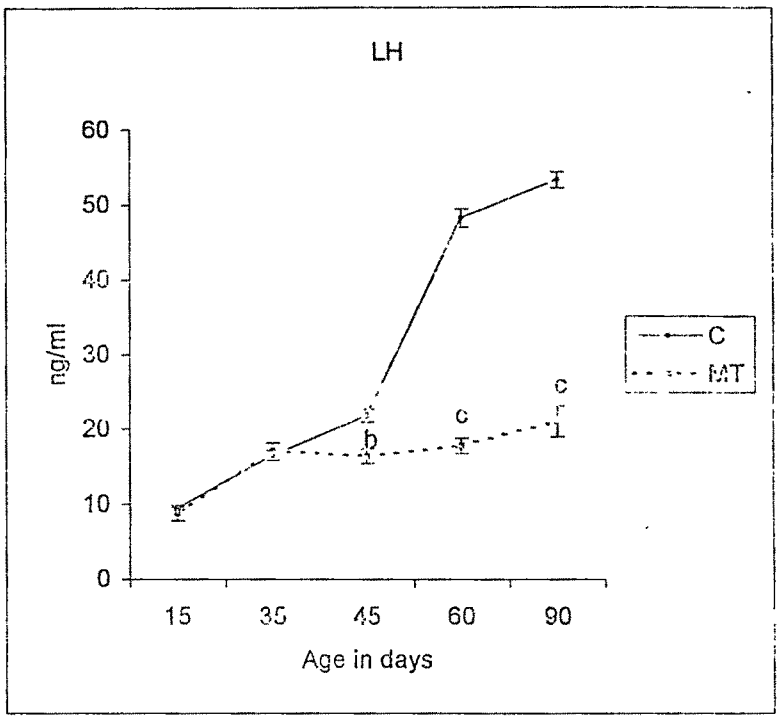
C - Control, **CE+MT** - Low Dose Corticosterone evening injection+Evening Melatonin injection Values expressed as Mean ± SEM of four samples [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

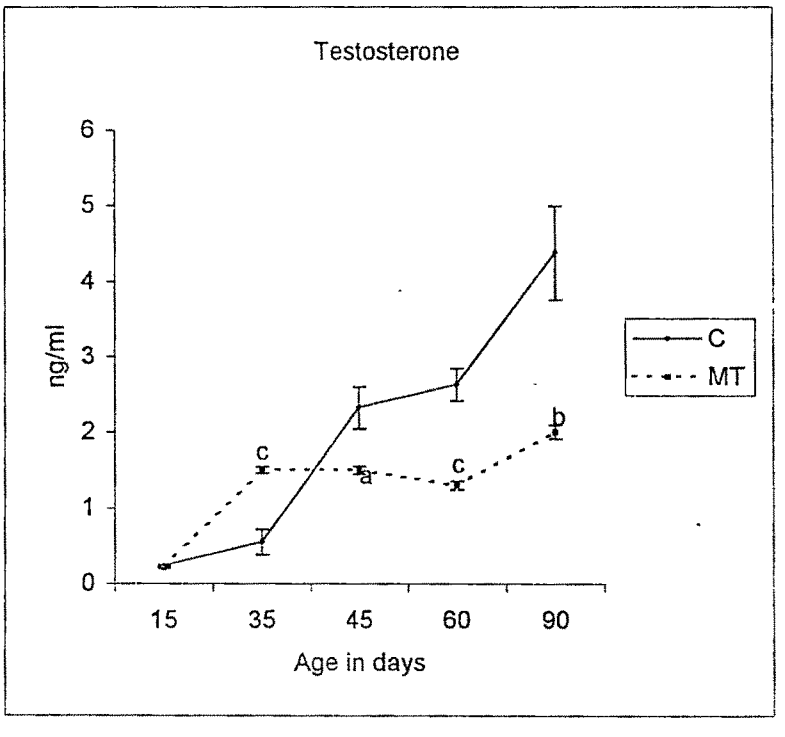




Serum LH and T levels (ng/ml) in Control and Corticosterone treated rats

C - Control, **CE** - Low Dose evening Corticosterone injection, **CM** - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of four samples, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$





Serum LH and T levels (ng/ml) in Control and Melatonin treated rats

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

Table 5: Serum TSH, T_4 and T_3 levels (ng/ml) in Control and Corticosterone + Melatonin treated rats

Treatment	TSH					T_4					T_3				
	Age in days					Age in days					Age in days				
	15	35	45	60	90	15	35	45	60	90	15	35	45	60	90
C	3.175 ±0.165	6.600 ±0.129	6.873 ±0 111	7.495 ±0.143	5.440 ±0.066	0.31 ±0.013	0.583 ±0.085	1.170 ±0.061	2.568 ±0.024	2.368 ±0.225	0.215 ±0.051	0.450 ±0.011	0.303 ±0.107	0.603 ±0.084	0.653 ±0.053
CE + MT	5 81 ^{c} ±0 043	7.5 ^{c} ±0.067	6.4 ^{b} ±0.079	5.8 ^{c} ±0.042	3.6 ^{c} ±0.021	1.01 ^{c} ±0 031	2.94 ^{c} ±0.016	3.52 ^{c} ±0.052	2.75 ^{b} ±0.026	3.8 ^{c} ±0.016	0.48 ^{c} ±0.019	0.51 ±0.050	0.42 ±0 024	0.42 ^{a} ±0.030	0.47 ^{a} ±0.035
*** MT**	14 01 ^{c} ±0 104	26 01 ^{c} ±1.577	9.050 ^{c} ±0 804	7.804 ^{c} ±804	9.001 ^{c} ±0.365	1.282 ^{c} ±0.084	1.700 ^{c} ±0.053	2.805 ^{c} ±0.015	2.212 ^{b} ±0.068	1.742 ^{c} ±0.018	0.400 ±0.026	0.481 ±0.056	0.800 ^{c} ±0.074	1.051 ^{c} ±0.084	0.910 ^{c} ±0.095
*** CE**	3 470 ±0.137	6 910 ±0 144	7.283 ±0.176	7 633 ±0.062	7.823 ±0.125	0.465 ±0.023	0.985 ±0.112	1.230 ±0.182	1.650 ^{c} ±0.074	2.468 ±0.225	0.305 ±0.039	0.583 ±0.031	0.734 ±0.086	1.053 ±0.056	0.400 ±0.082

C – Control, **CE + MT** - Low Dose Corticosterone evening injection + Evening Melatonin injection, **CE** - Low Dose Corticosterone evening injection **MT** - Evening Melatonin injection

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

* Values recalled from Chapters 1 and 2

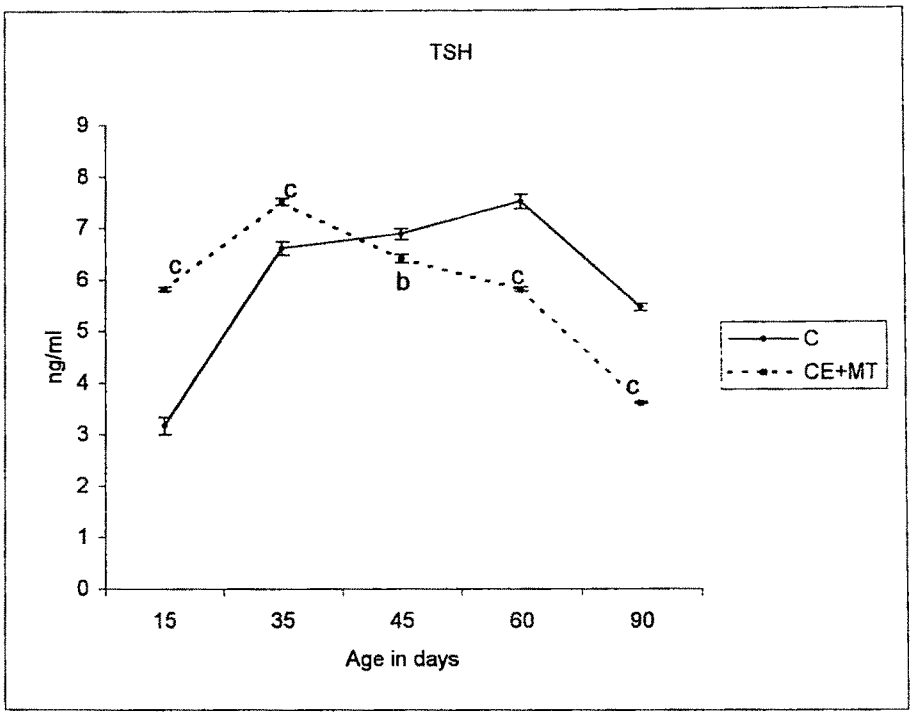
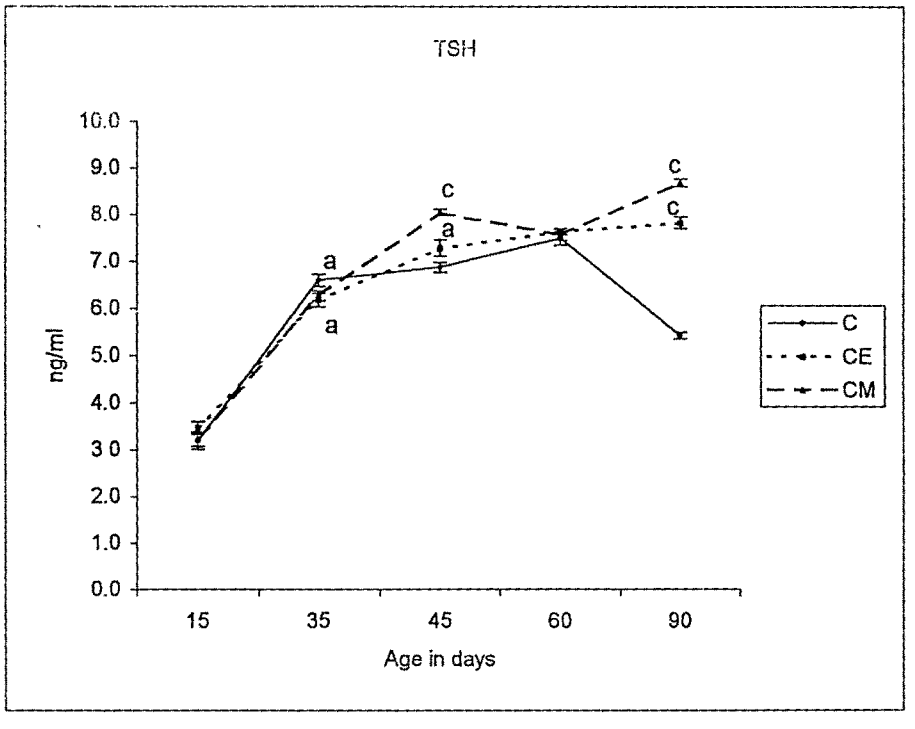
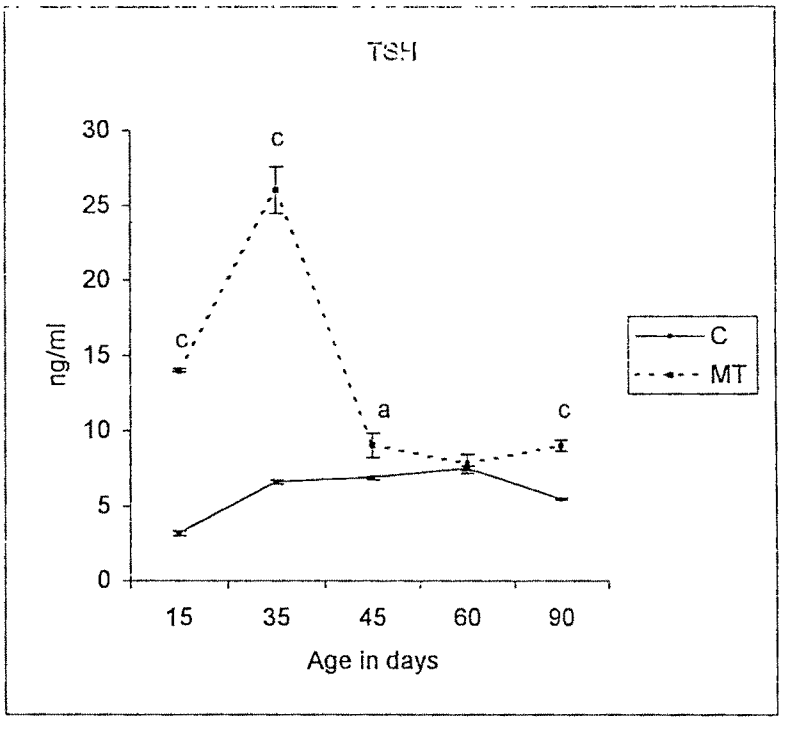


Fig. 6: Serum TSH (ng/ml) level in Control and Corticosterone + Melatonin treated rats

C - Control, **CE+MT** - Low Dose Corticosterone evenin injection+Evening Melatonin injection, Values expressed as Mean ± SEM of four samples [a] $p< 0.05$, [b] $p< 0.005$, [c] $p< 0.0005$



Serum TSH level (ng/ml) in Control and Corticosterone treated rats
C - Control, **CE** - Low Dose evening Corticosterone injection, **CM** - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of four samples, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

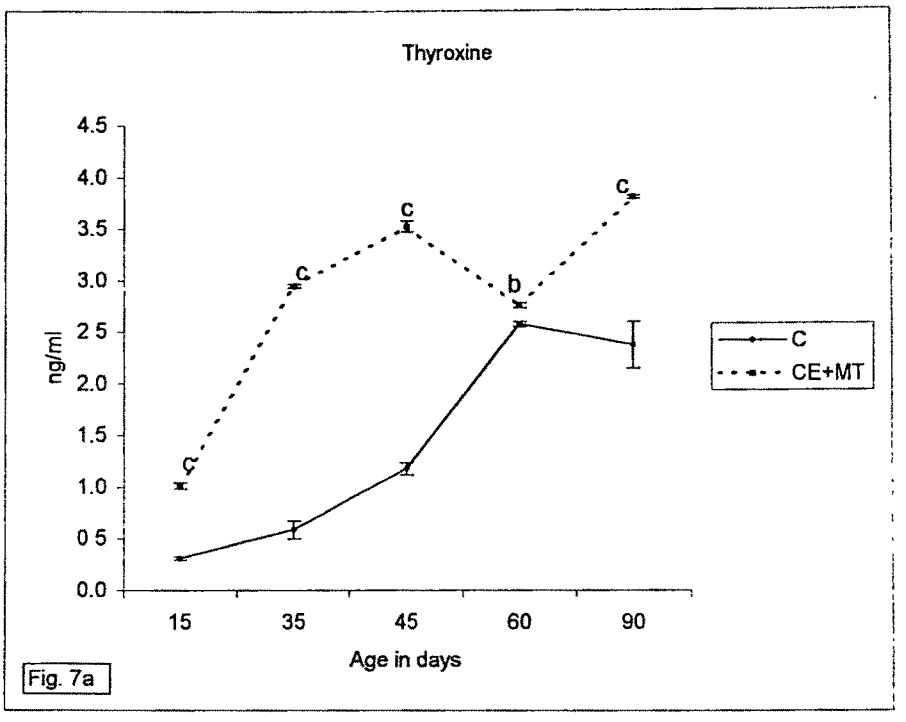


Serum TSH level (ng/ml) in Control and Melatonin treated ra

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$



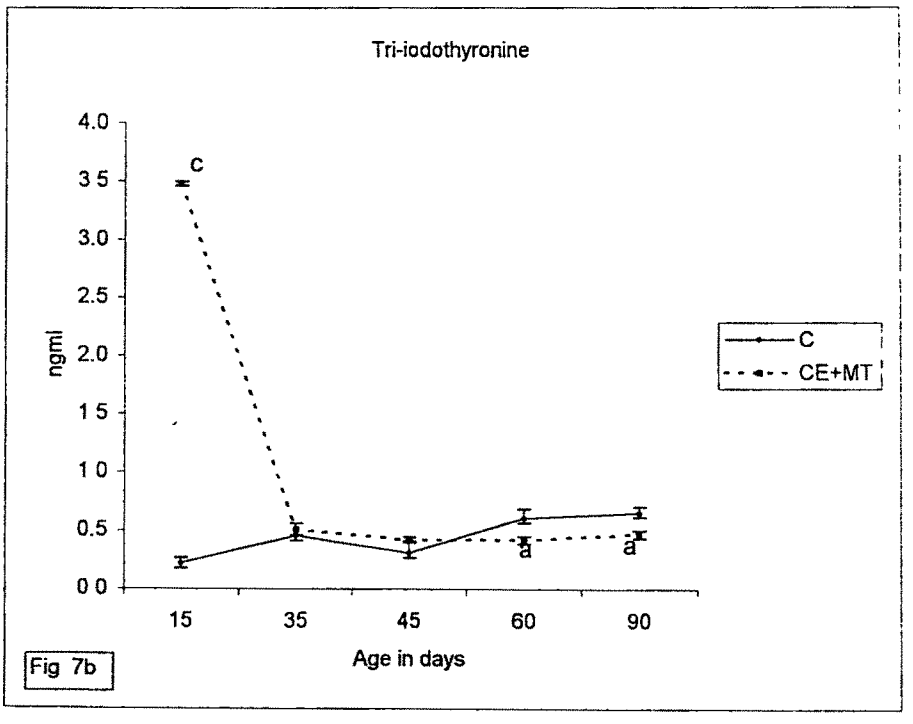
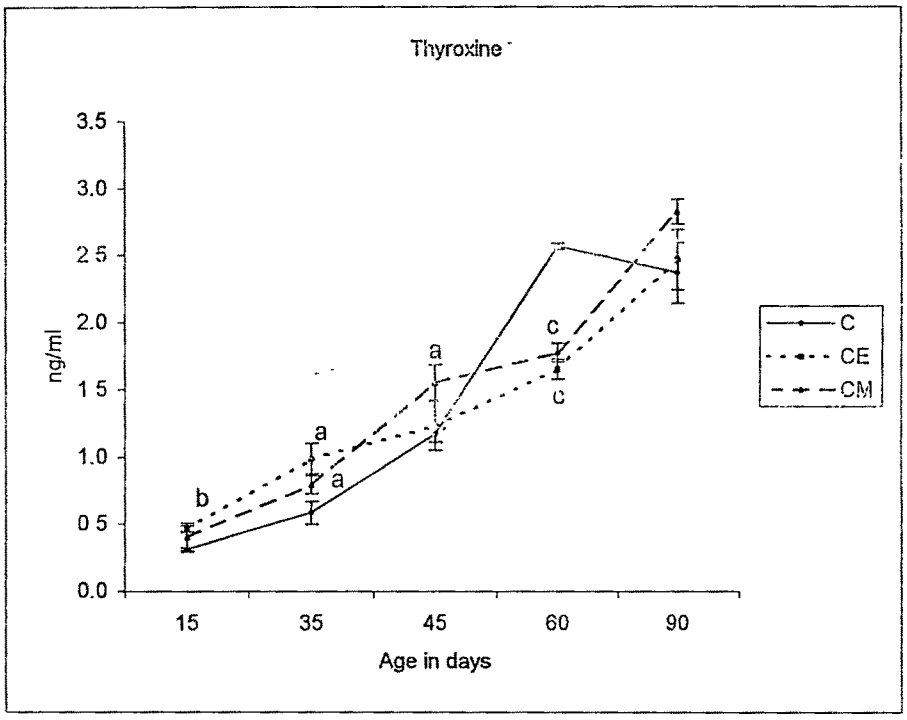
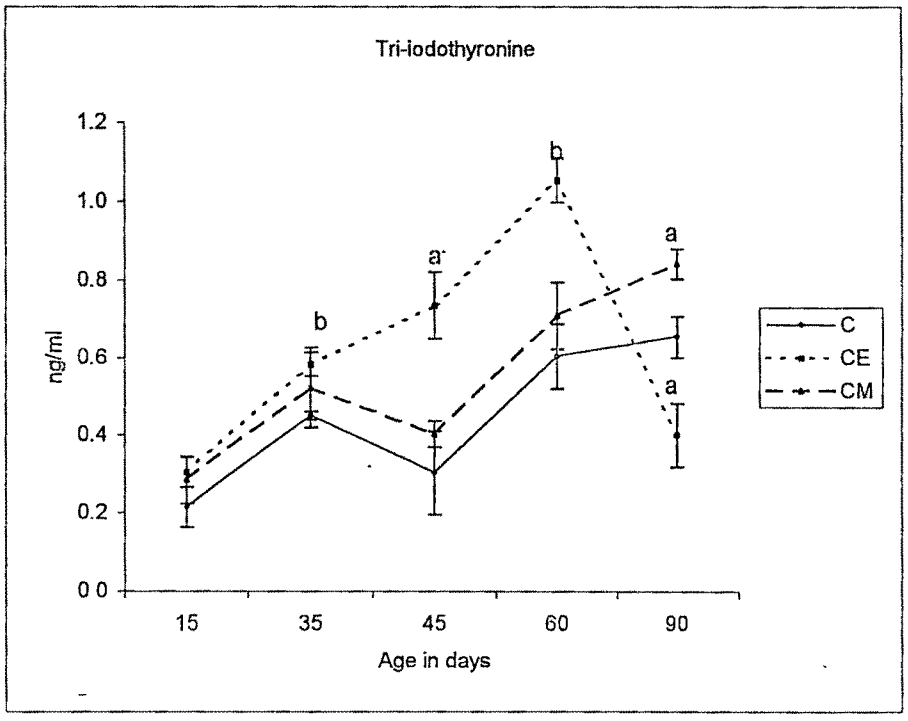


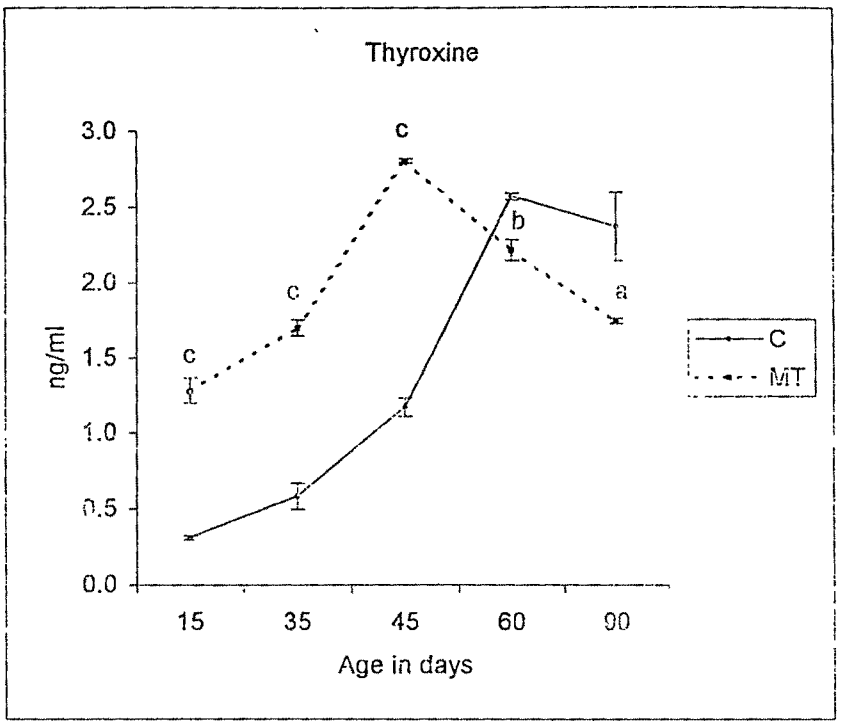
Fig. 7a and 7b: Serum T_4 and T_3 (ng/ml) levels in Control and Corticosterone + Melatonin treated rats.
C - Control, **CE+MT** - Low Dose Corticosterone evenin injection+Evening Melatonin injection, Values expressed as Mean ± SEM of four samples [a] $p< 0.05$, [b] $p< 0.005$, [c] $p< 0.0005$





Serum T_4 and T_3 levels (ng/ml) in Control and Corticosterone treated rats

C - Control, **CE** - Low Dose evening Corticosterone injection, **CM** - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of four samples, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$



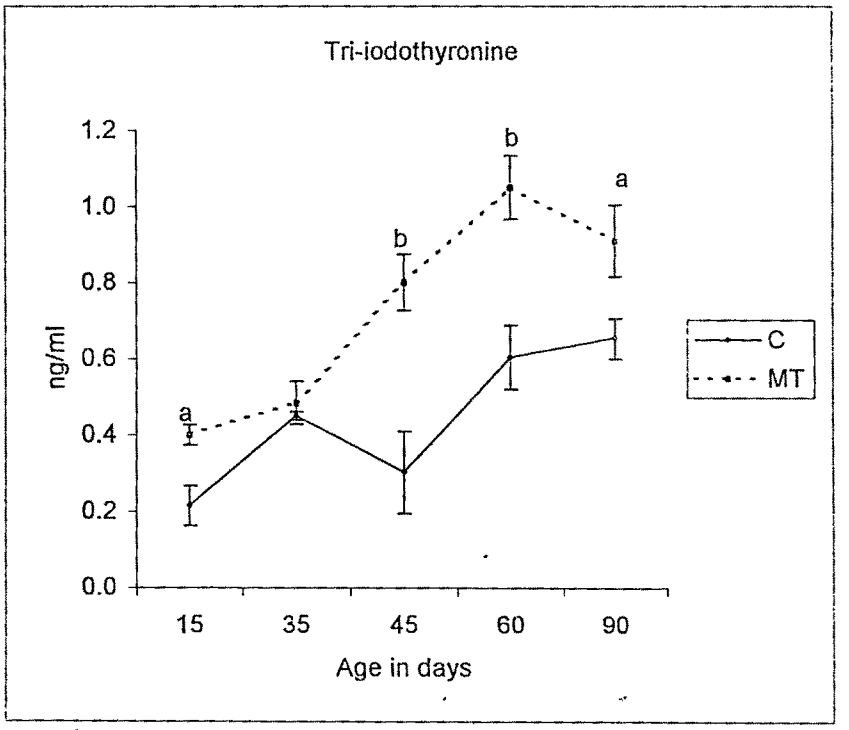


Fig.7a and 7b: Serum T_4 and T_3 levels (ng/ml) in Control and Melatonin treated rats.

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

treatment period (35 days). Whereas the levels of TSH and T_3 registered their maximal levels at 35 days, the level of T_4 reached its highest at 45 days. The levels of TSH and T_3 were decreased and were significantly subnormal thereafter (45, 60 and 90 days). However, T_4 showed significantly higher level than controls at all ages (Table 5; Figures 6, 7a & 7b).

LH and Testosterone:

Both LH and T showed a continuous increase in control animals from the lowest level at 15 days to a maximum at 90 days. In the case of CE+MT animals also, a similar trend of increase was noticed till 60 days, but with significantly higher levels corresponding to the age matched controls. Between 60 and 90 days, the levels of both the hormones decreased significantly to control level in the case of LH and, in the case of testosterone below the control level (Table 4; Figures 5a & 5b).

DISCUSSION:

The present study involving a combination of hypercorticalism and hypermelatonemia in the neonatal period has shown remarkable deleterious changes on spermatogenesis without affecting the testes weight and, altered neuroendocrine axes of thyroid, adrenal and gonad. Previous studies showed that neonatal evening corticosterone has favourable influence on puberty onset and spermatogenesis and hastened body and testes growth (Chapters 1 and 2). The above study also showed increased growth rate of the body

upto 60 days and of testes upto 45 days in the corticosterone treated animals. The subsequent decline in growth rate was significantly and perceptibly lower than in the controls. In another study, evening melatonin treatment in the neonatal period was shown to register continuous increment in body growth rate till 90 days and of testes till 60 days with the result, the body and testes weight of these rats were significantly higher at 90 days (Chapter 1). Interestingly, CE+MT animals showed body growth changes intermediary to those of CE and MT animals, and testes growth changes similar to those of melatonin animals indicating complex interactive influence of the two hormones. The melatonin effect on testes growth is reflected in the lesser relative weight at 90 days but the interactive influence of melatonin and corticosterone is indicated by the higher than melatonin relative weight but lower than CE relative weight (Table 1).

The inferred melatonin effect on testis is further validated by the observed degenerative and apoptotic germ cell loss during prepubertal and pubertal periods and germ cell loss combined with premature detachment of spermatids and spermatozoa in the adult. These changes were seen at a perceptibly greater degree in melatonin treated animals (Chapter 1), but not in corticosterone treated animals (Chapter 2). In fact, transient neonatal corticosterone excess was inferred to be favourable for spermatogenesis (quantitatively and qualitatively) by decreasing germ cell apoptosis and leading to tightly packed compact germinal epithelium by promoting better

association between germ cells and Sertoli cells. But it is clear from the present observations that the favourable influence of corticosterone is completely nullified by melatonin and in fact the deleterious effect of melatonin is potentiated in a corticosterone background. The large-scale denudation of germinal epithelium by the enmass detachment of the round and elongated spermatids leaves the tubules with very little sperm. The influence of melatonin is further revealed by the length of the tubules at 35 days, increased germ cell loss compared to CE and, similar high germ cell degeneration as in MT. The greater germ cell loss is indicated by the increased theoretical count, with no increase in the actual count.

Number of reasons could be sought in explaining the quantitatively and qualitatively deleterious effect on spermatogenesis observed in the present study,

1. A supra optimal level of corticosterone during the first five weeks of neonatal period (infantile and juvenile) might in fact have an opposite damaging influence on germinal epithelium compared to an optimal level as seen in our previous study on neonatal corticosterone excess (Chapters 1 and 2). Both melatonin treated rats as well as CE+MT rats have higher levels of corticosterone at these periods with maximal levels in the combination treatment (Table 3), which could be the *raison detre*.

2. It could also be a case of up-regulation of corticosterone receptors or, sensitivity to melatonin or, vice versa, on Sertoli and/or germ cells, which could be responsible for the observed detrimental effect on spermatogenesis.

3. Another speculative explanation could be the decreased FSH and/or testosterone responsiveness of the seminiferous tubules under the melatonin-corticosterone combination. Whatever be the reason(s), the mediated effects seem to be a long-term one probably brought about by permanent genetic reprogramming of the Sertoli and germ cells. The possible consequential effect on the expression of Sertoli cell and germ cell adhesion molecules could be of significance and recent reports are in the process of identifying many candidate molecules (D'Agostino *et al.,* 1984; D'Agostino and Stefanini, 1990; Pratt *et al.,* 1993; Vera *et al.,* 1993; Lampa *et al.,* 1999; Maeda *et al.,* 1999).

The neonatal corticosterone-melatonin excess also seems to affect the endocrine reproductive axis as the circulating titres of LH are pronouncedly higher throughout and the testosterone titres significantly lower in the post pubertal periods in relation to LH levels. In fact, the testosterone level at 90 days is significantly lower in CE+MT as well as, melatonin and, CE animals, corresponding to the age matched controls. It may be speculated that the experimental

hormonal combination in the neonatal period has a permanent central set point elevating influence by decreasing the sensitivity of GnRH pulse generator and the pituitary to the negative feedback action of the gonadal steroids. Further, the Leydig cells also seem to be depicting reduced responsiveness to LH despite the presence of prominent Leydig cells. Such an influence of melatonin in lowering the sensitivity of Leydig cells to LH have been seen in many *in vivo* and *in vitro* studies (Lang *et al.,* 1983, 1984; Valenti *et al.,* 1995; Vanecek, 1999). The hypothalamo-hypophyseal-adrenal (HHA) axis is also down regulated, as the corticosterone levels are significantly subnormal in the post pubertal and adult stages. This seems to be a corticosterone effect as, the observed levels are quite similar to those observed in our previous work on neonatal corticosterone excess (Table 4). Melatonin seems to be incapable of negating this effect of corticosterone as, melatonin treatment did not alter the adult corticosterone levels. There seems to be a very characteristic effect on the hypothalamo-hypophyseal-thyroid (HHT) axis as, postpubertal TSH and T_3 levels are significantly reduced while, the T_4 levels are increased. The pituitary insensitivity to TRH seems to be reduced while the sensitivity of thyroid to TSH seems to be increased with either decreased T_3 output or attenuated peripheral conversion. Clearly, the corticosterone-melatonin combination effect is quite distinct and may suggest a new

homeostatic thyroid axis, affected by the synergistic modulatory influence of neonatal melatonin and corticosterone excess.

In conclusion, it could be said that neonatal corticosterone-melatonin excess has deleterious unfavourable influence on adult testis functions, a potentiated influence of melatonin on a corticosterone background and, permanent long-term alterations in the thyroid, adrenal and gonad neuroendocrine axes. It is also inferable that the neonatal period is a sensitive period and that various hormonal principles have interactive modulatory influence on the maturation of the neuroendocrine axes.

PLATE – I

Figures 1 – 8: Photomicrographs of sections of testis of control rats.

Figures 1 and 2 : Sections of testis of 35 day old control rats showing interstitium.

Figures 3 and 4 : Section of testis of 45 day of showing advanced stages of spermatogenesis and appearance of sperms in few tubules.

Figures 5 and 6 : Section of testis of 60 day old rats showing well-established spermatogenesis and sperms in lumen.

Figures 7 and 8 : Section of testis of 90 day old rats showing prominent interstitium and fully established spermatogenesis.

Figures: 1, 3, 5, & 7 – 250 x
Figures: 2, 4, 6, & 8 – 400 x

Abbreviations:

I-Interstitium, **L**-Lumen, **st**-spermatids, **S**-sperms,
D-Degeneration,**rs**-round spermatids.

REVERSE PLATE

PLATE – VII a

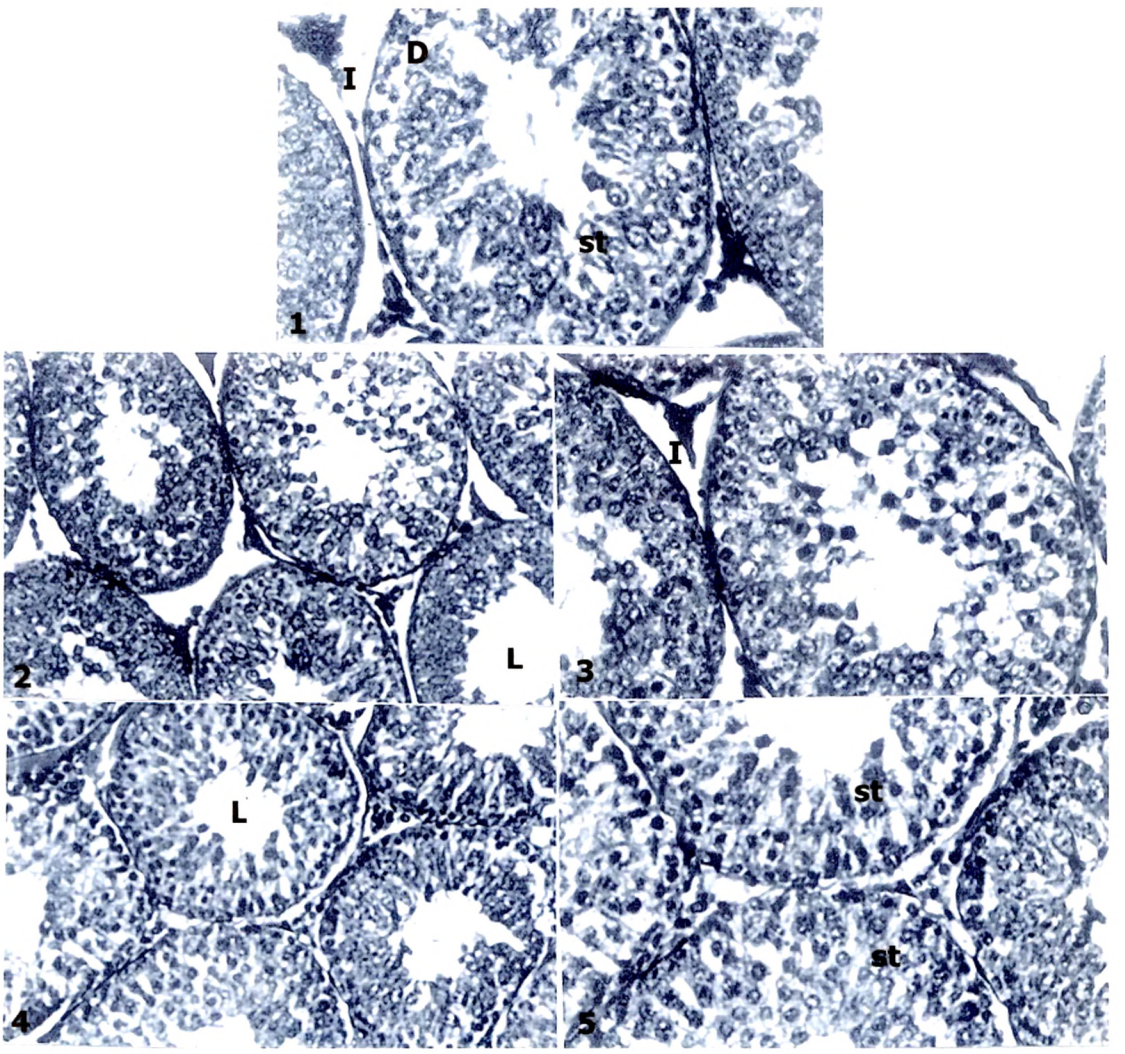
Figures 1 – 5: Photomicrographs of sections of testis of rats treated with corticosterone and melatonin.

Figures 1 to 3 : Sections of testis of 35 day old CE+MT rats showing, elongated spermatids and degenerative changes in germ cells.

Figures 4 and 5 : Section of testis of 45 day old CE+MT rats showing cellular dislodgement and reduced degenerative changes (as compared to 35 day old)

CE+MT – Evening Corticosterone + Melatonin injection

Figures: 2 and 4 – 250 x
Figures: 1, 3 and 5 – 400 x


I
D
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1
2
L
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I
4
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5
st
st

PLATE – VII b

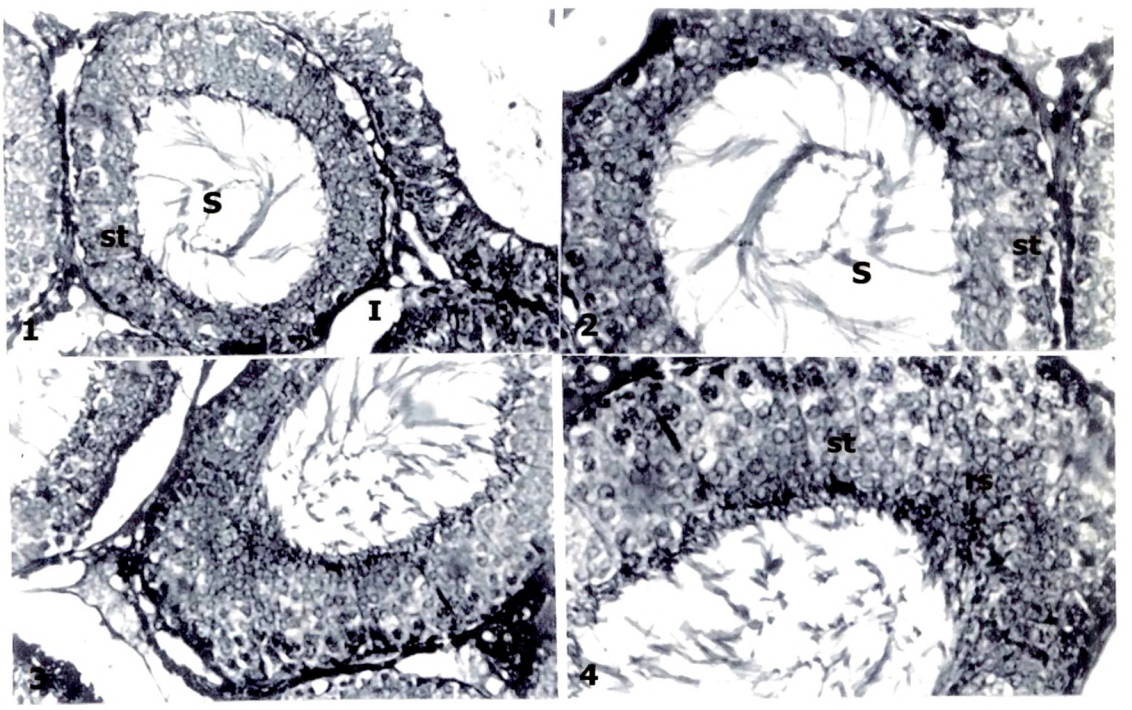
Figures 1 – 4: Photomicrographs of sections of testis in rats treated with corticosterone and melatonin.

Figures 1 and 2 : Sections of testis of 60 day old CE+MT rats showing, less dense mass of spermatids and germ cells and sperms seen.

Figures 3 and 4 : Section of 90 day old testis in CE+MT rats showing still reduced degeneration, very poor sperm mass and more dislodgement.

CE+MT – Evening Corticosterone + Melatonin injection

Figures: 1 and 3 – 250 x
Figures: 2 and 4 – 400 x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4

SUMMARY

Previous studies have found that small doses of corticosterone within physiological range in the neonatal period are favourable for early testes maturation when given in the evening, and that melatonin (MT) administration has many paradoxical effects on testis function and on various endocrine axes, all of which are either due to indirect modulation by corticosterone or by a direct action of melatonin or even, interactive actions between melatonin, corticosterone and testosterone. In the present study, the influence of evening melatonin in neonates rendered hypercorticalic by evening administration (Low dose) of corticosterone from day 0 to day 21, had been evaluated in terms of adult testes weight, spermatogenic and steroidogenic functions and hormonal profiles in the postweanling period till adulthood. The favourable influence of corticosterone is completely nullified by melatonin and in fact the deleterious effect of MT is more potentiated in a corticosterone background, as large scale sloughing off of round and elongated spermatids leaves the tubules with very little sperm. This is indicated by the normal number of germ cells countable as against increased theoretical count thereby contributing to a higher percentage of germ cell loss. This damaging effect is attributed to either higher levels of corticosterone in the neonatal periods, or up regulation of corticosterone receptors or, sensitivity to melatonin or vice versa on Sertoli and/or germ

cells, or, decreased FSH and/or testosterone responsiveness of seminiferous tubules under the melatonin-corticosterone combination. It is also speculated that the hormonal combination in the neonatal period has permanent central set point elevating influence of decreasing the sensitivity of GnRH pulse generator and of the pituitary to the negative feedback action of gonadal steroids. The hypothalamo-hypophyseal-adrenal (HHA) axis is down regulated probably, a 'corticosterone excess' effect and MT is incapable of negating it. There is a very characteristic effect on hypothalamo-hypophyseal-thyroid (HHT) axis, as postpubertal TSH and T_3 levels are significantly reduced while T_4 level is increased, a synergistic modulatory influence of neonatal melatonin and corticosterone excess. It can be concluded that a combined evening neonatal corticosterone-melatonin excess potentiates the unfavourable melatonin effect in a corticosterone background.