CHAPTER - V

Neonatal morning corticosterone excess further potentiates the detrimental effects of melatonin on spermatogenesis: II. Time dependent effect

Adult reproductive functions especially gonadal steroidogenesis, are known to be adversely affected by elevation in glucocorticoids either due to disease, stress or experimental administration in animals and man (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). Foetal exposure to corticosterone has also been reported to affect postnatal reproductive development as well as puberty onset in both sexes (Harvey and Chevins, 1987; Politch and Herrenkohl, 1994; Smith and Waddell, 2000). Previously, we had examined the effect of corticosterone excess in the neonatal preweanling period in the rat and found it to be favourable for hastening testes maturation when given in the evening (Chapter 2). In another study, similar experimental paradigm with doubled dose of corticosterone, though found to have similar favourable influence on germ cell number and onset of spermatogenesis, was nevertheless found to induce premature loss of spermatids and spermatozoa (Chapter 3). Studies involving similar dosage

regimen in the morning also seem to produce responses similar to evening administration though with lesser degree of favourable manifestations and with slightly higher deleterious effects (Chapters 2 & 3). Melatonin has been reported to delay reproductive development in both male and female rats when administered in the pre-pubertal ages (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 inhibitory effect of melatonin on sexual maturation of rat has also been demonstrated when administered in the afternoon between 20-40 days of age (Lang *et al.,* 1983). No effect of melatonin was seen by the above workers during melatonin administration between 5 and 20 days of age or during 70 and 90 days of age (Lang *et al.,* 1983). Though a delay in sexual maturation was observed due to melatonin treatment between 20 and 40 days of age, these animals were found to be normal as adults in terms of reproductive functions (Lang *et al.,* 1983). A time independent influence of melatonin (morning or evening) on both body and testes growth was reported from this laboratory when the hormone was given between 10 to 25 days of age (Patel and Ramachandran, 1992). A recent study evaluated the long-term influence of exogenous melatonin during the first three weeks of neonatal life on adult testis size, structure and function and found favourable influence in the form of increased germ cell number and an unfavourable response in the form of loss of advanced germ cells (Chapter 1). The previous studies on both neonatal corticosterone

excess as well as neonatal melatonin excess revealed effects on the hypothalamo-hypophyseal adrenal, thyroid and gonad axes, apart from the effects on testis functions (Chapters 1,2,3 & 4).

In the wake of these observations it was thought worthwhile to evaluate the influence of simultaneous excess of corticosterone and melatonin on postnatal growth and maturation of testis as well as its functions in the adult. To this end, a combination of both melatonin and corticosterone was administered in the evening to rat neonates between day 0 to day 21 and, was demonstrated to have more deleterious effects than with melatonin treatment alone (Chapter 1). In the present study, a different experimental regimen consisting of morning corticosterone and evening melatonin has been employed to test the effect of this differentially timed combination on adult body and testes weight, spermatogenic and steroidogenic functions and adult hormonal axes.

MATERIAL AND METHODS:

Animals and Maintenance:

As in chapter one.

Preparation of Corticosterone and Melatonin:

As in chapters one and two.

Experimental Protocol:

The experimental set-up was divided into two major groups of study, boyh 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 **(N)**

(ii) Injected *i.p.* with vehicle (0.9% saline) in morning (0800 hrs) + 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.

(i) Melatonin injections (*i.p.*) were given in the evening (1600 hrs), and CORT injections (morning - High dose) were given in the morning (0800 hrs) **(CM+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:

At 90 day, the body weight of CM+MT animals was slightly higher and that of testes significantly lower. Similar body and testes weights were recorded during the treatment period as well as the immediate post-treatment period (35 days) in both control and experimental animals (Table 1; Figures 1a & 1b). However, both the body and testes weights showed significant increase in the experimental animals at 45 and 60 days. These changes in weights are clearly marked by the recorded growth rates (Table 2; Figures 3a & 3b). Peak growth rate of both body and testes was recorded between 45-60 days in the control rats while the same was recorded between

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 CM has been recalled from chapters 1 and 3 respectively.

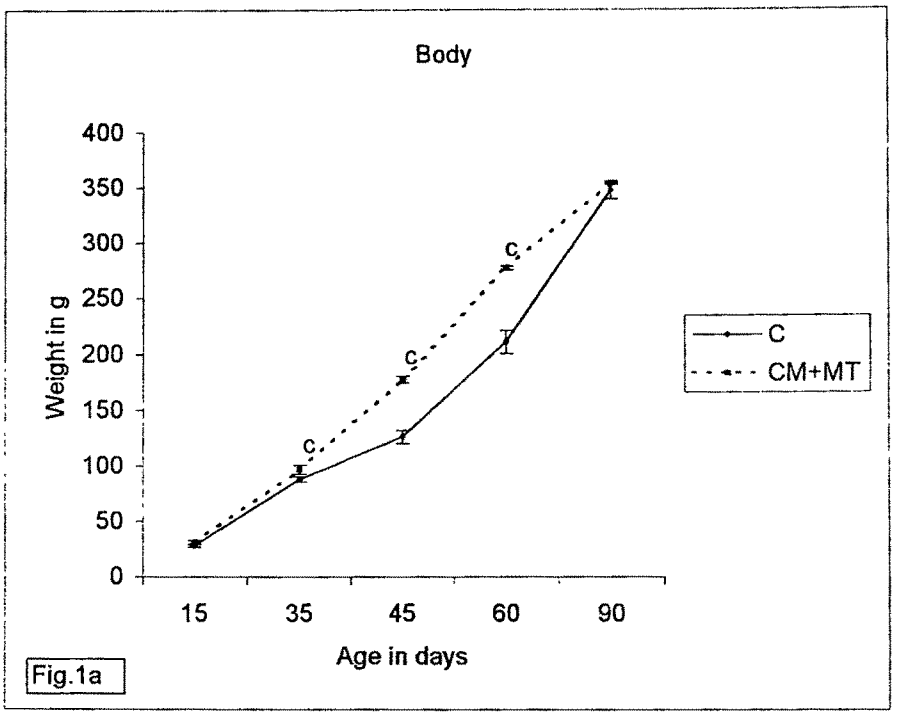
Table 1: Chronological alterations in body weight (g) and absolute (g) and relative weight (g/100 g) of testes in Control and Corticosterone + Melatonin treated rats

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	27.6 ±1.38	87.43 ±2.54	125.7 ±6.23	210.8 ±10.43	345.97 ±7.421	0.21 ±0.011	0.871 ±0.062	1.204 ±0.092	2.553 ±0.075	3.289 ±0.224	0 67 ±0.021	0.894 ±0.033	1.097 ±0.027	1.260 ±0.033	0.954 ±0.030
CM+MT	30 5 ±1.86	96.388^{c} ±4.206	177.0^{c} ±3.090	277.0^{c} ±1.592	353.0 ±1.5209	0.198 ±0.014	0.854 ±0.035	1.800^{c} ±0.065	2.840^{a} ±0.057	3.074 ±0.055	0.66 ±0.027	0.89 ±0.036	1.06 ±0.035	1.03^{c} ±0.016	0.79^{c} ±0.006
* C	37.33 ±1 919	85.513 ±1.902	117.167 ±2.307	193.688 ±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	152.33^{c} ±2.486	233 66^{c} ±3.242	396.66^{c} ±9.898	0.213^{a} ±0.003	0.938 ±0.054	2.214^{c} 0.102	2 214^{a} ±0.102	3.375^{c} ±0.078	0.687 ±0.001	0.854 ±0.004	1.11 ±0.42	1.16 ±0 063	0.847 ±0.043
C	29 98 ±1 645	94 16 ±3.804	±129.69 ±3.667	206.4 ±6 369	336.15 ±6.280	0.291 ±0.015	0.956 ±0.078	1.48 ±0.58	2.52 ±0.85	3 17 ±0.104	0 970 ±0 056	1.02 ±0.064	1.41 ±0.083	1.22 ±0.067	0.943 ±0.046
* CM	35.5^{a} ±1.89	115.5^{c} 0.671	173.6^{c} ±1.763	267.8^{c} ±3.301	315.8^{a} ±3.270	0.199 ±0.02	1.069 ±0.009	1.81 0.056	2.79 ±0.032	3.01 ±0.050	0.536^{c} ±0.036	0.998^{a} ±0.079	1.003 ±0.082	1 031^{a} ±0.076	0.983 ±0.083

C – Control, **CM+MT** - High Dose Corticosterone evening injection + Evening Melatonin injection, **CM**- 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 3



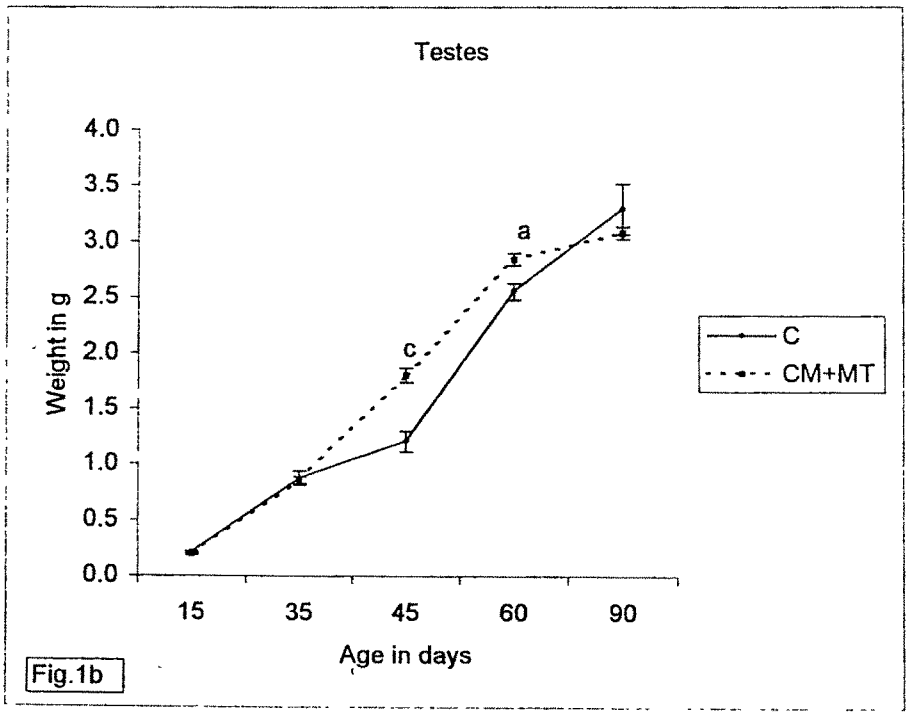
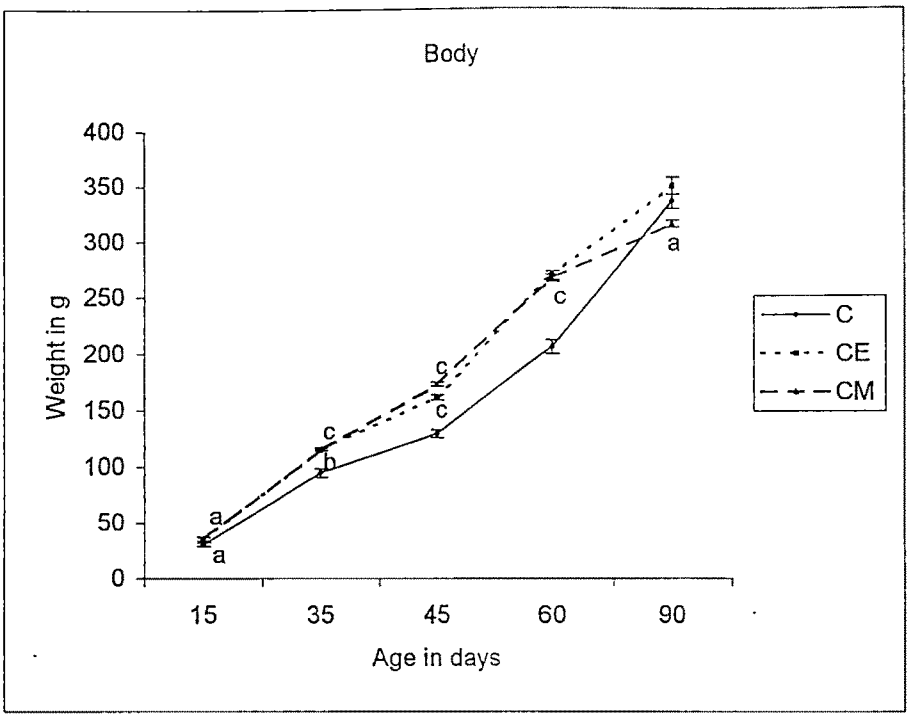
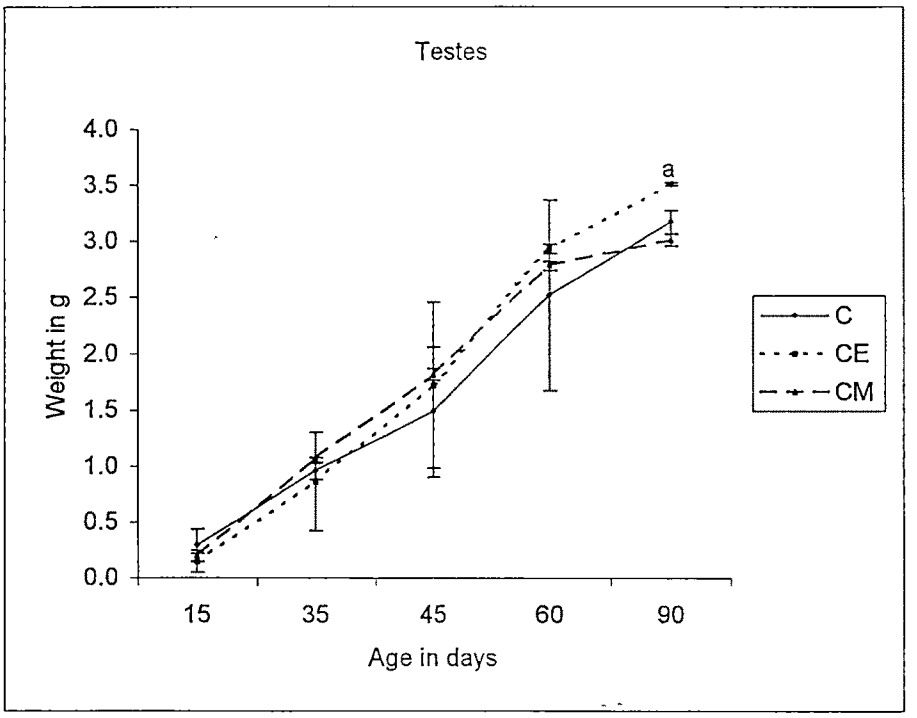
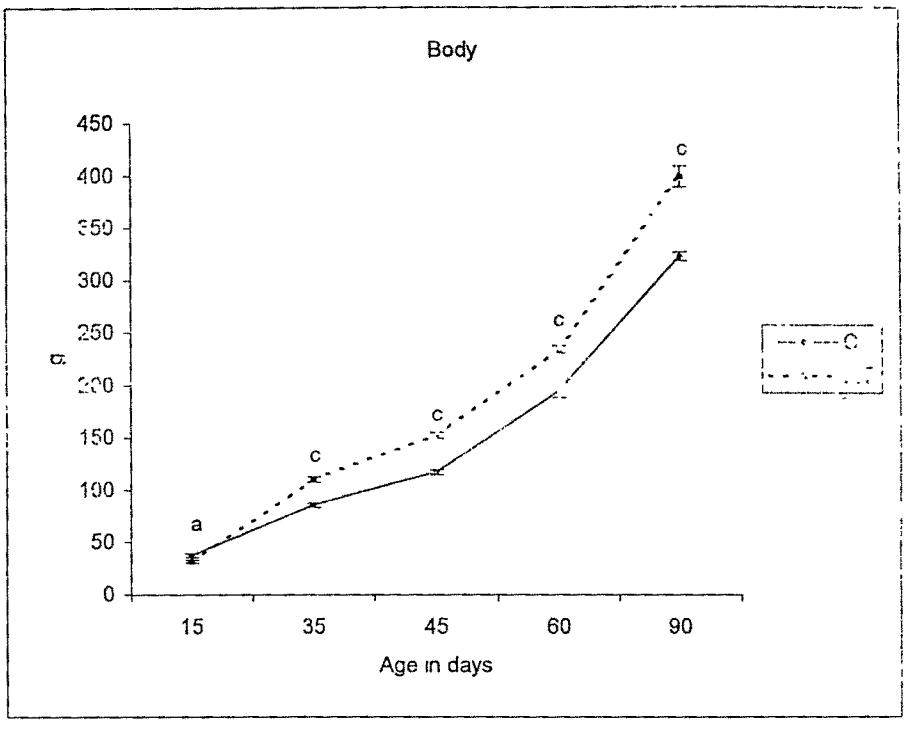


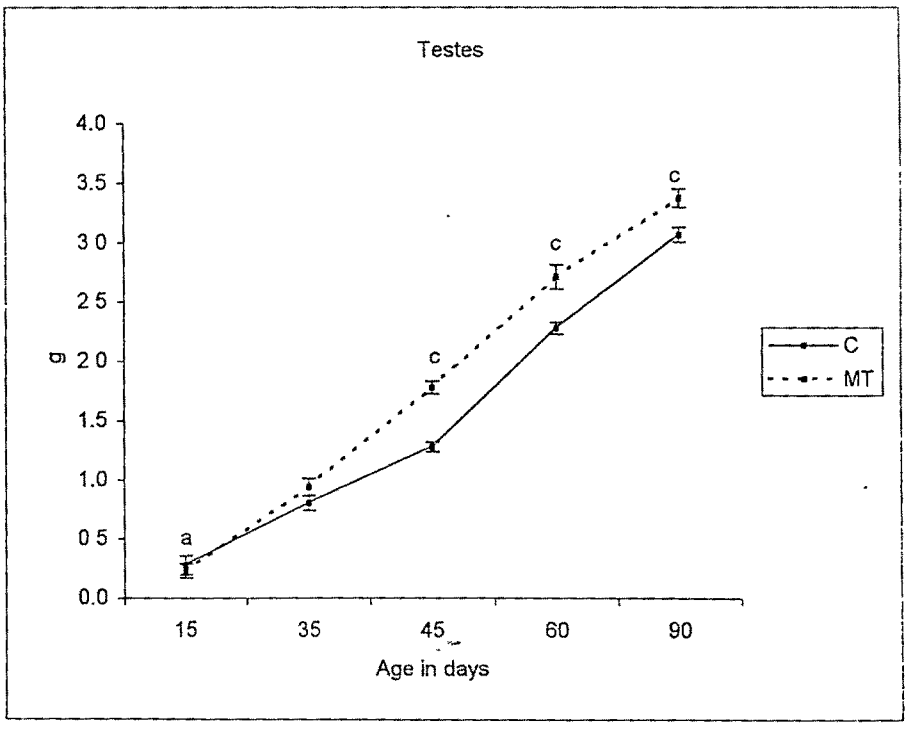
Fig. 1a and 1b. Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Corticosterone + Melatonin treated rats **C** - Control, **CM+MT** - High Dose Corticosterone evening injection+Evening Melatonin injection, Values expressed as Mean ± SEM of six animals , [a] $p< 0.05$, [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** - High dose evening Corticosterone injection, **CM** - High dose morning Corticosterone injection, Values expressed as Mean ± SEM of six animals [B89]





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] $p < 0.0005$

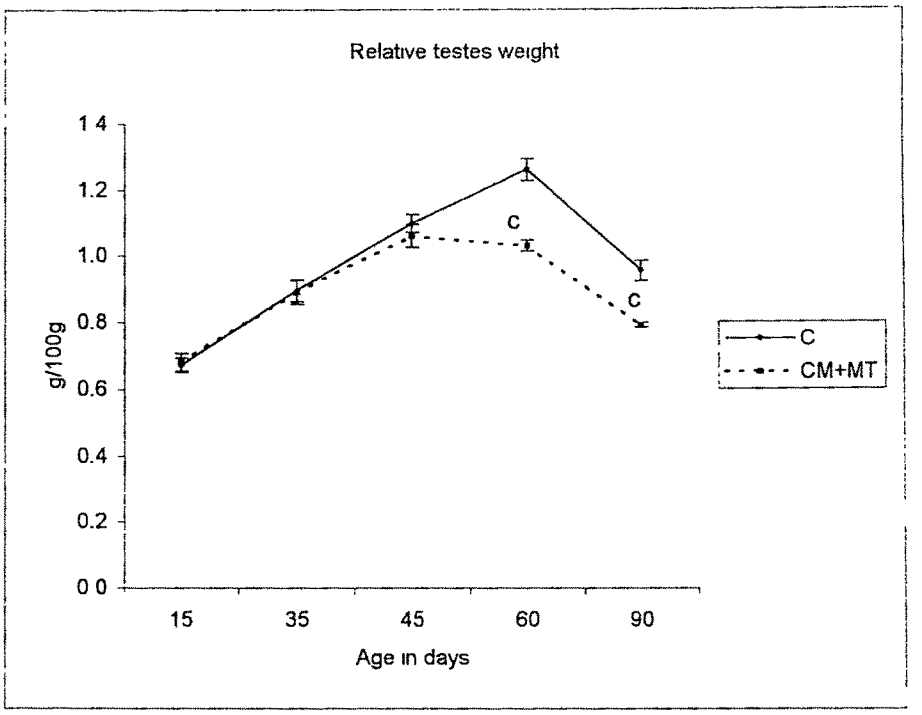
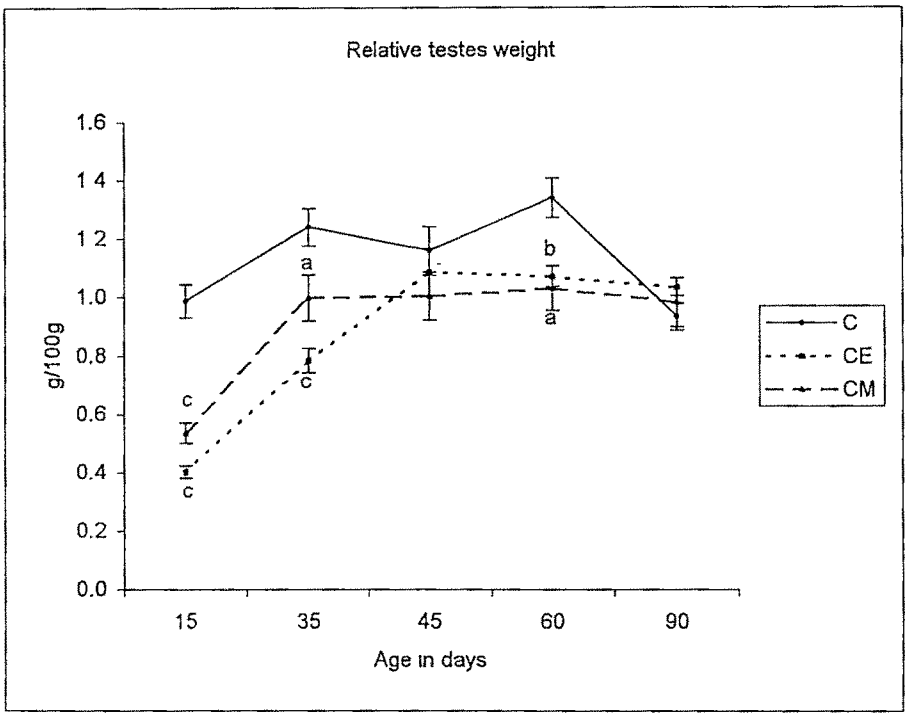
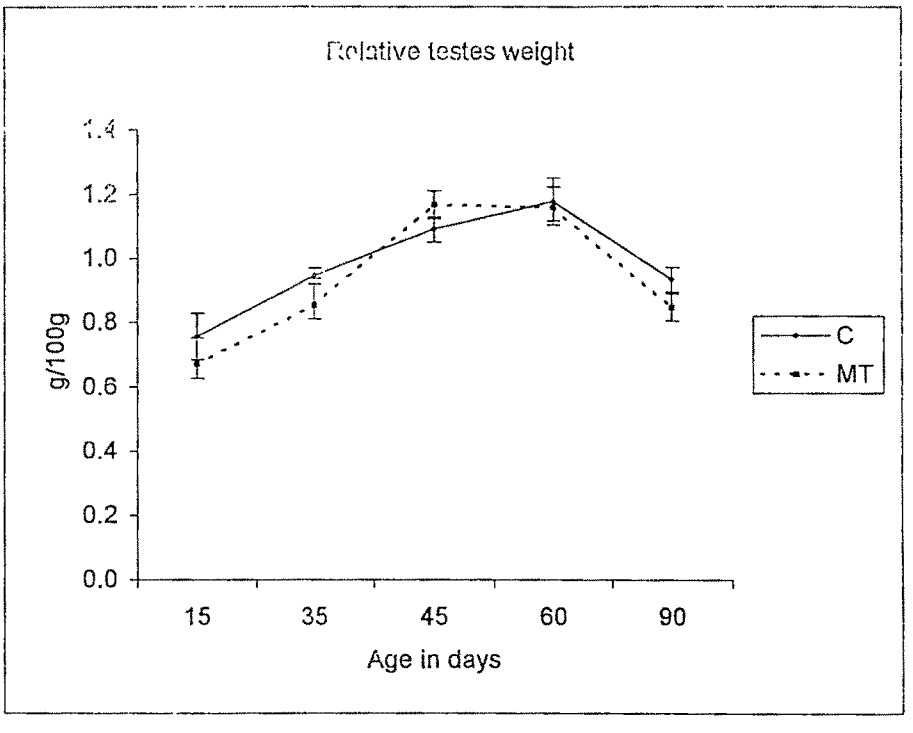


Fig.2: Chronological alterations in relative weight (g/100 g) of testes in Control and Corticosterone + Melatonin treated rats
C - Control, **CM+MT** - High Dose Corticosterone evening injection+Evening Melatonin injection, Values expressed as Mean ± SEM of six animals
[a] $p < 0.05$, [b] $p < 0.005$,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



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

C - Control, **CE** - High dose evening Corticosterone injection, **CM** - High 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

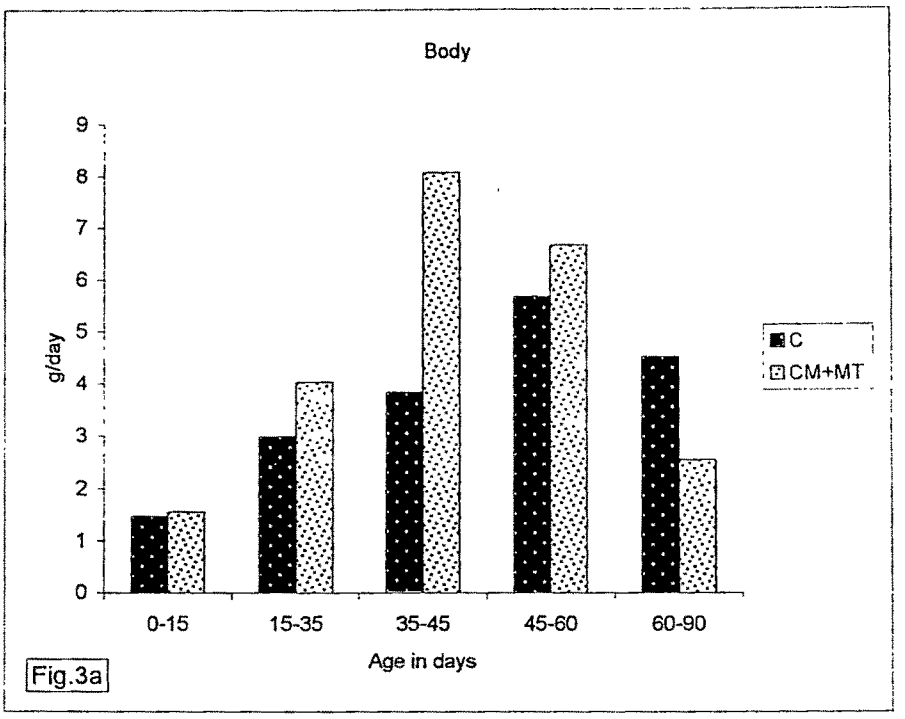
Values expressed as Mean ± SEM of six animals [a] $p< 0\ 05$, [b] $p< 0\ 005$, [c] $p< 0.0005$

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	1.47	2.992	3.827	5.673	4 50	0.014	0.033	0 033	0.090	0.025
CM+MT	1.550	4.031	8.061	6 667	2.533	0 013	0 047	0 095	0.069	0 008
* MT	1.663	3 942	4 200	5.422	5 500	0 015	0 035	0 084	0.062	0 022
* CM	1.84	3.85	5.8	6.28	1.60	0.013	0.043	0 074	0.065	0 007

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

* Values recalled from Chapters 1 and 3



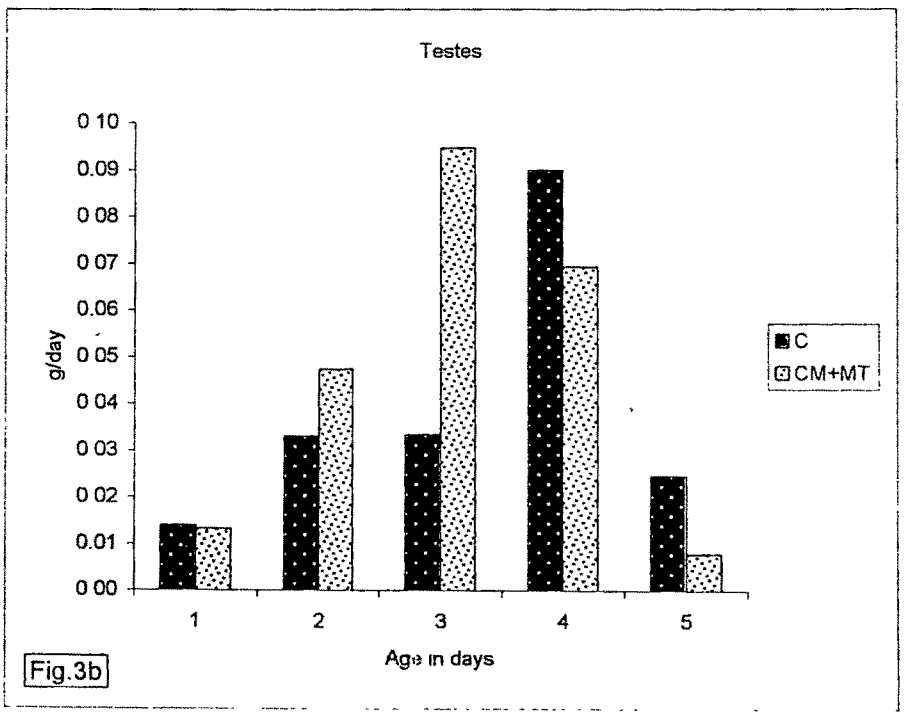
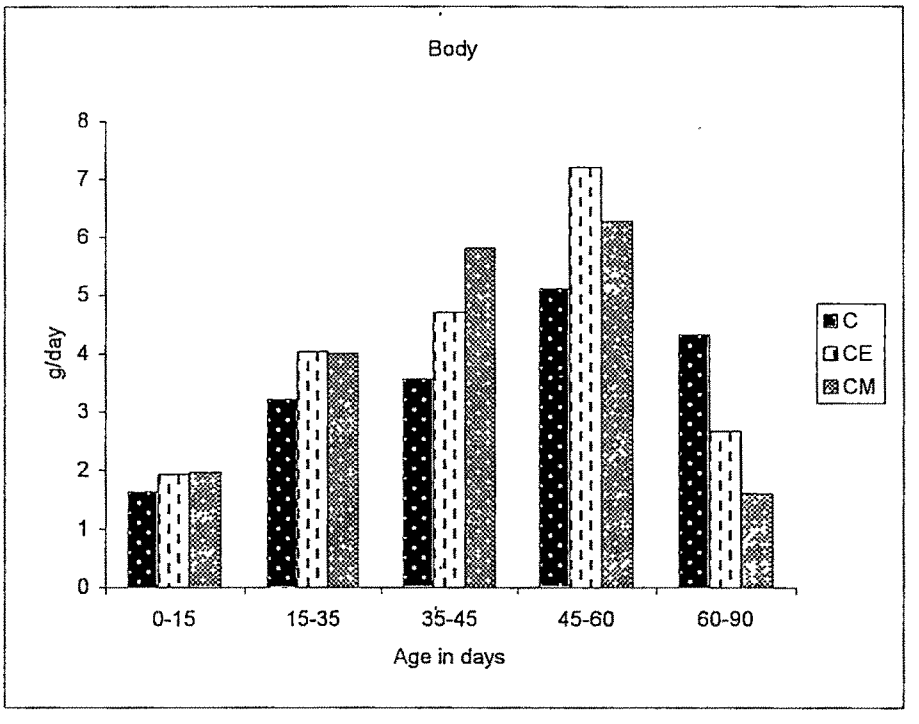
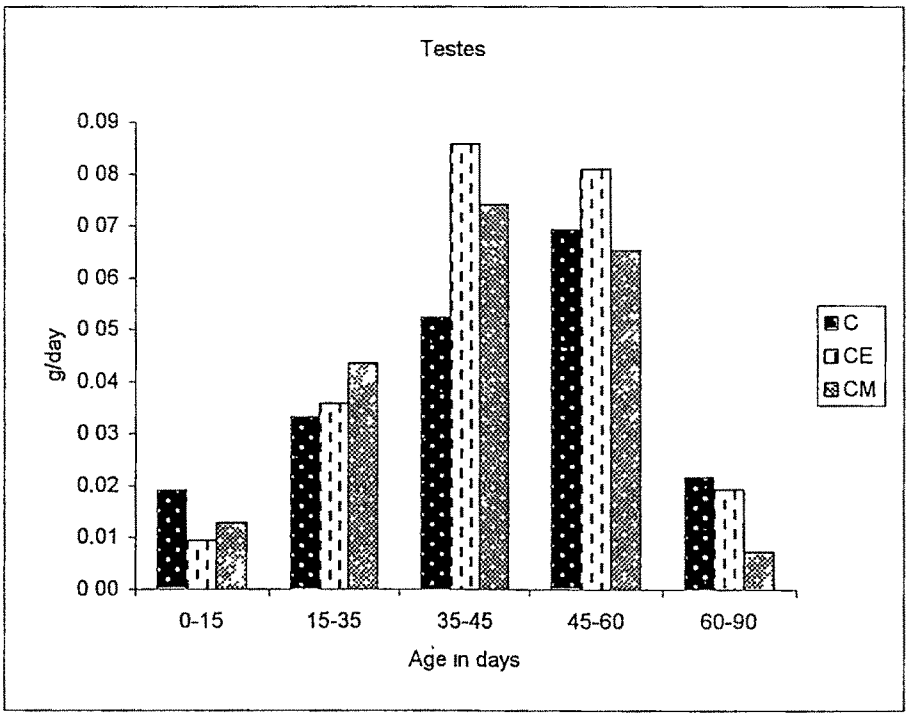


Fig. 3a and 3b: Per day Body and Testes Growth rate (g/day) in Control and Corticosterone + Melatonin treated rats

C - Control, **CM+MT** - High Dose Corticosterone evening injection+Evening injection, Values expressed as Mean ± SEM of six animals

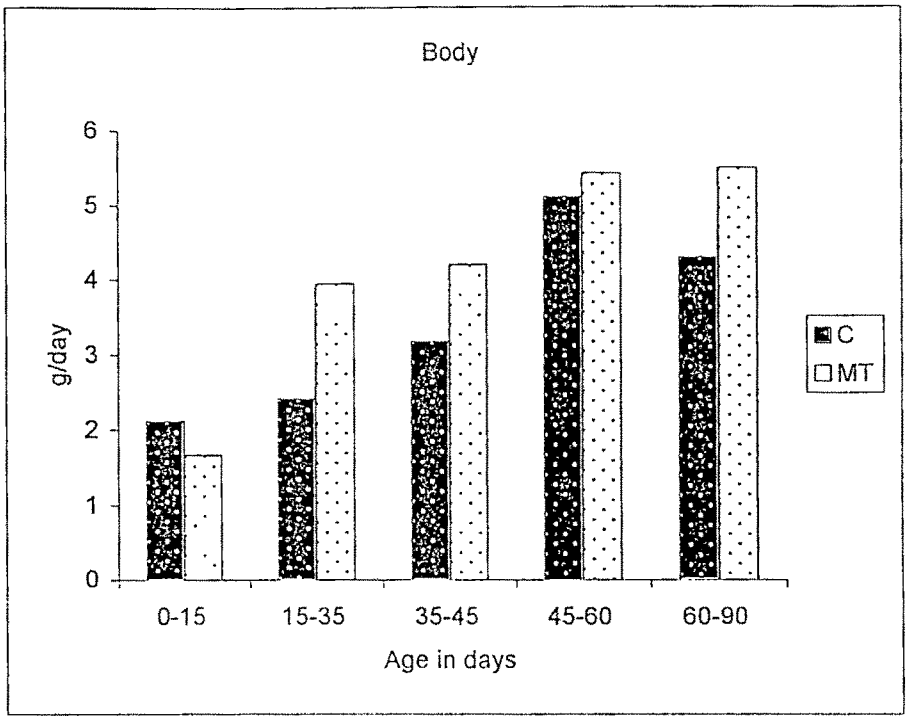
[a] **p< 0.05,** [b] **p< 0.005,** [c] **p< 0.0005**

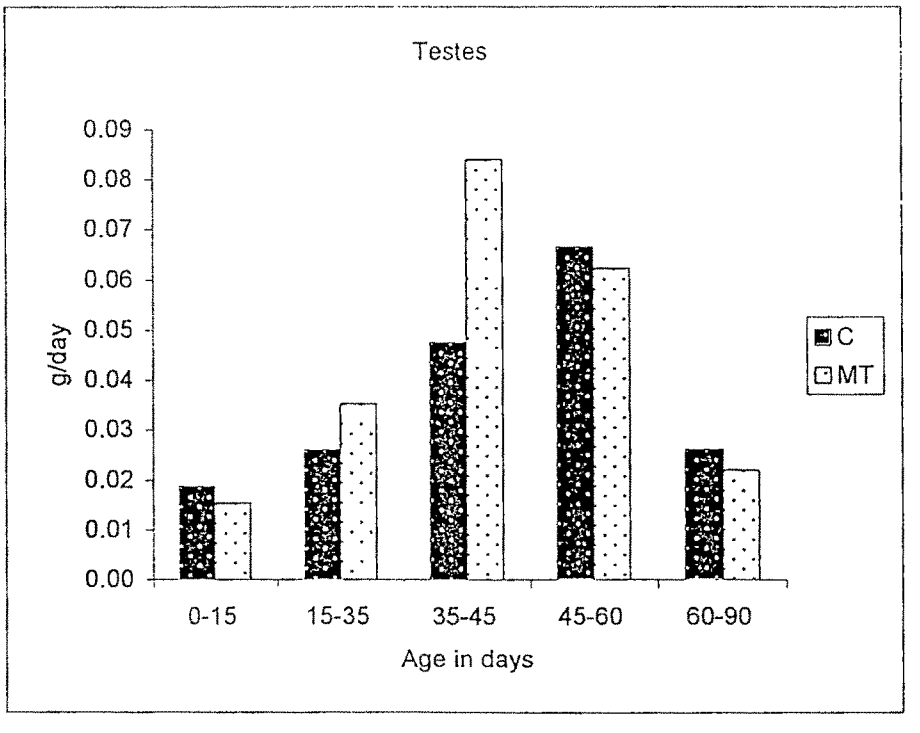




Per day Body and Testes growth rate (g/day) in Control and Corticosterone treated animals

C - Control, **CE** - High dose evening Corticosterone injection, **CM** - High dose morning Corticosterone injection





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

35 and 45 days in the experimental animals. The decrement in both body and testes growth rate occurring between 60 and 90 days was significantly pronounced in CM+MT rats. The weight of testes expressed relative to body weight was found to be lower in CM+MT animals throughout with increasing percentage difference compared to controls between 35 and 90 days (Table 2; Figure 2).

Histology and Histometry:

The diameter of seminiferous tubules and the germinal epithelium were both less than the control at 35, 45 and 60 days, but at 90 days they both were greater than the controls, changes comparable to melatonin treated animals. Spermatogenesis was fully established by 45 days marked by the appearance of sperms. However, the germ cell density was lower and the cells were found to be loosely arranged. At 60 and 90 days extensive degeneration and premature detachment of spermatids and spermatozoa were visible with marked luminal accumulation of the cells and disruption of germ cells towards the lumen. Over all, the sperm density was very poor. The testicular and tubular volume at 90 days, were both, lesser by 6-7% compared to controls. The length of seminiferous tubules per testis and the total basement area were both lesser than in controls by 21 % and 14 % respectively. The increase of these two parameters at 90 days from the measurements in the 35-day testis was only 30.4% and 117% respectively in

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
CM+MT	1.403 ±0.080	0.0310^c ±0.0004	0.0087^c ±0.0001	1.347 ±0.060	1832.07^c ±56.900	176.123^c ±2.600	25.648^b ±1.400	308.0 ±2.900	230.0^c ±3.900	16.81^c ±0.290	12.55 ±0.980	25.34^c ±0.300
* MT	1.541 ±0.070	0.033^c ±0.001	0.0100 ±0.0002	1.448 ±0.060	1725.16^c ±45.30	177.205^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
* CM	1.374 ±0.040	0.0259 ±0.0090	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

C – Control, **CM+MT** – High Dose Corticosterone morning 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 3

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.

the experimentals as against 140% and 234% in the controls. These are similar to the observations made in melatonin treated animals. The total Sertoli cell count showed a 21% decrement from the control. Whereas the change in Sertoli cell number was similar to that in melatonin rats, the germ cell number was similar to that recorded in CM (Table 3) (Plates I, II, VIIIa & VIIIb).

Serum Hormone Profile:

Corticosterone:

The serum corticosterone titre was significantly higher in CM+MT animals at 15, 35 and 45 days but then decreased to significantly lower levels at 60 and 90 days (Table 4; Figure 4). These changes in corticosterone levels were similar to the levels recorded in CM animals.

TSH, T_4 and T_3:

In general, TSH level was significantly higher during the treatment as well as post-treatment periods in CM+MT animals (Table 5; Figure 6). However, T_4 level was higher at 15, 35 and 45 days but significantly lower at 60 and 90 days and the T_3 level tended to remain significantly lower than the controls at 60 and 90 days but higher only during the treatment period. At 35 and 45 days the levels were comparable to controls (Table 5; Figures 7a & 7b).

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	45.55 ±1.699	9.390 ±0.132	16.450 ±0.634	21.75 ±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 ±0.620
CM+MT	18 42^{c} ±1.67	35.0^{c} ±3.24	19.7^{b} ±1.96	25.7^{c} ±1.25	19.0^{c} ±2.67	7.1 ±1.64	9 6^{b} ±1.18	10.9^{b} ±1.96	24.0 ^{c} ±2.67	18.0 ^{c} ±2.35	0.29^{a} ±0.004	1.0^{a} ±0.005	1.36^{b} ±0.06	1.7 ±0.09	2.0 ±0.06
*** 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
*** CM**	34 1^{c} ±2.04	28.8^{c} ±2.80	21.0^{c} ±1.76	18.7^{c} ±1.065	15.6^{c} ±1.76	15.56^{b} ±1.65	21.9^{a} ±2.61	13.15^{b} ±2.01	31.44^{c} ±1.78	31.3^{c} ±1.64	0.48^{c} ±0.021	2.40^{c} ±0.099	1.60^{a} ±0.076	2.80 ±0.085	1.30^{c} ±0.032

C – Control, **CM+MT** - High Dose Corticosterone evening injection + Evening Melatonin injection, **CM** - High 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 3

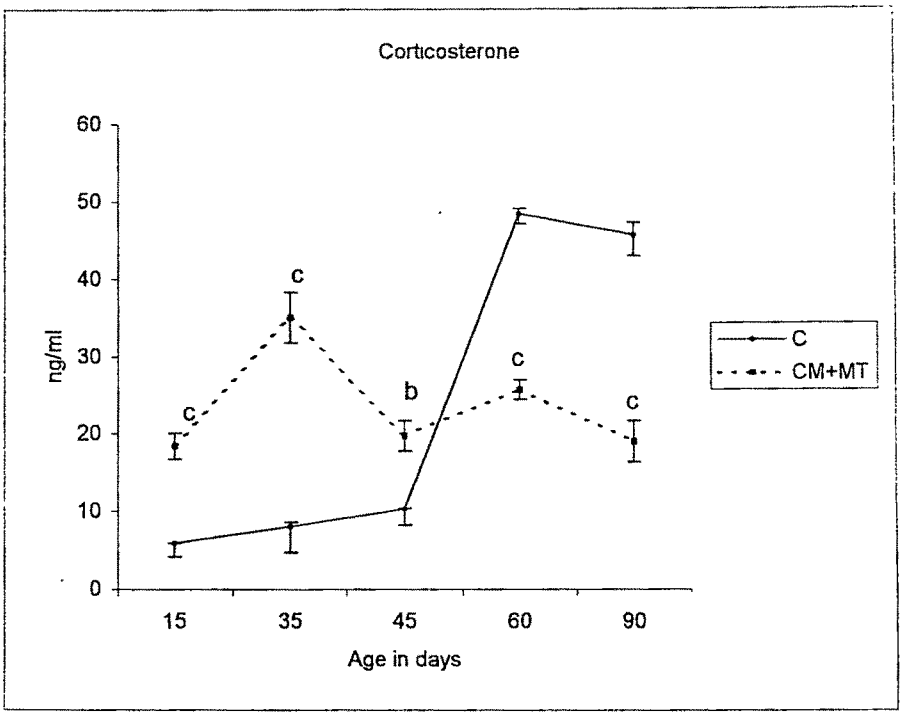
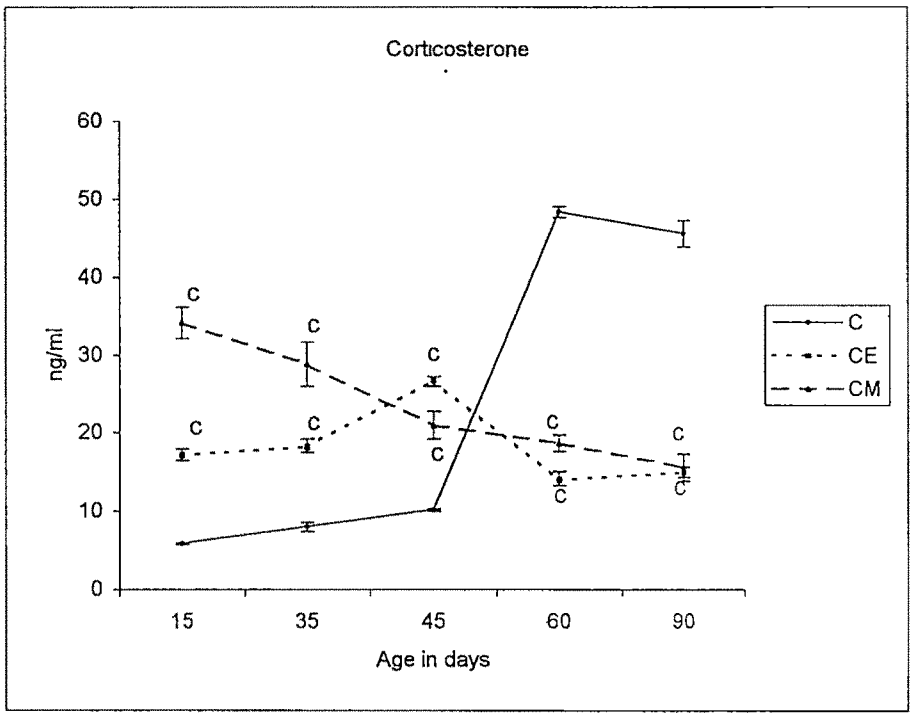


Fig.4: Serum Corticosterone level (ng/ml) in Control and Corticosterone + Melatonin treated rats

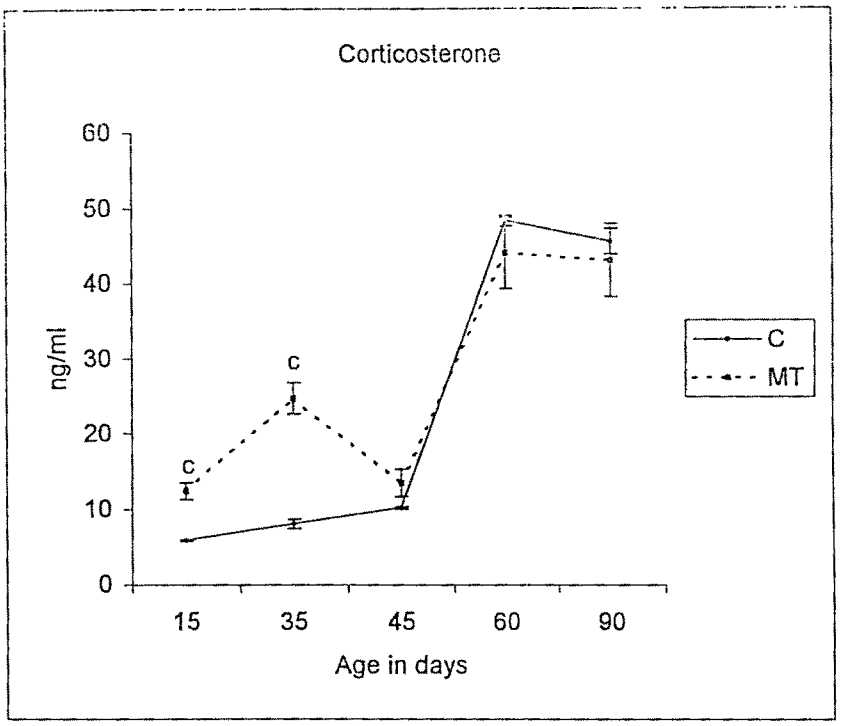
C - Control, **CM+MT** - High Dose Corticosterone evening injection + Evening Melatonin injection, Values expressed as Mean ± SEM of four samples

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



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

C - Control, **CE** - High dose evening Corticosterone injection, **CM** - High 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 $p < 0.0005$

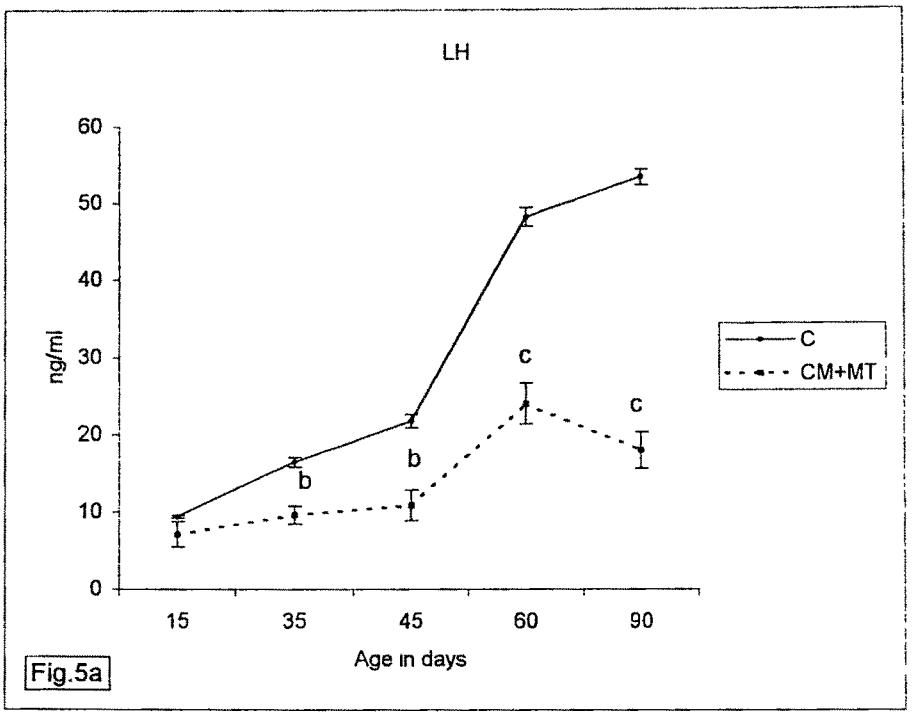


Fig.5a

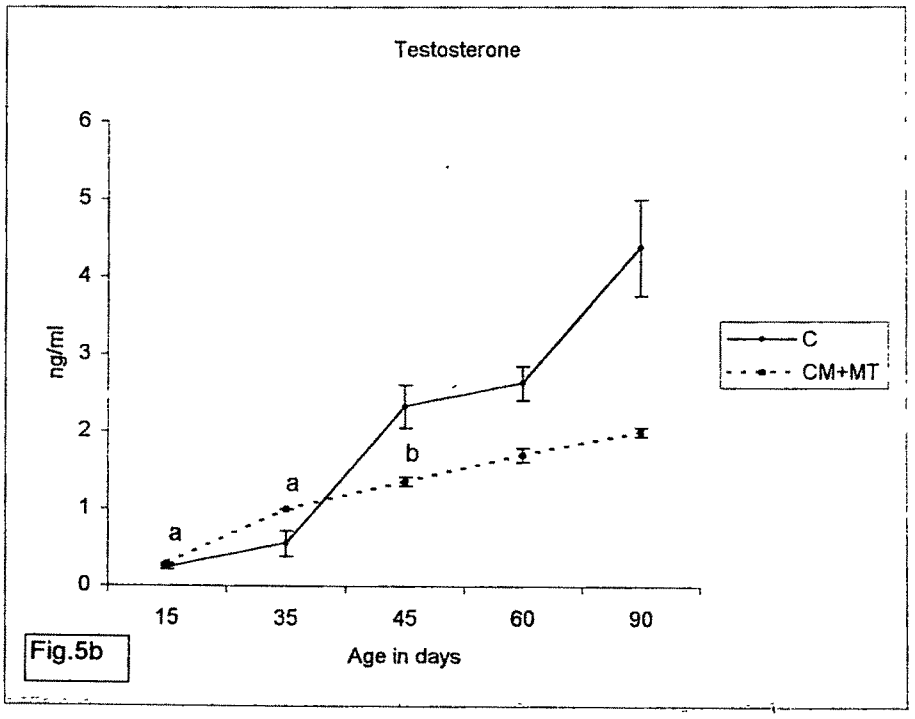
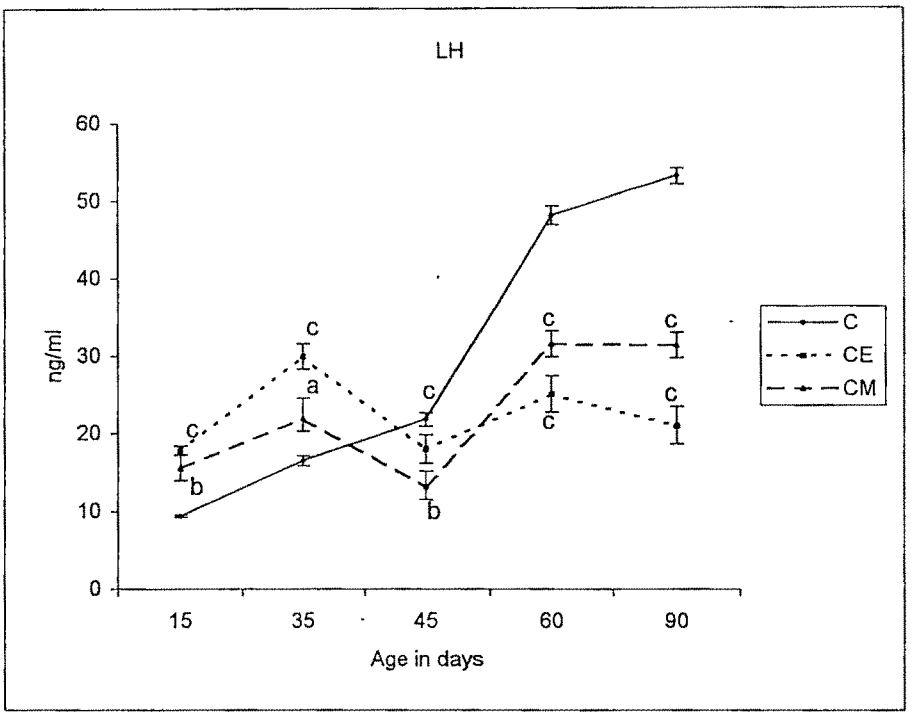


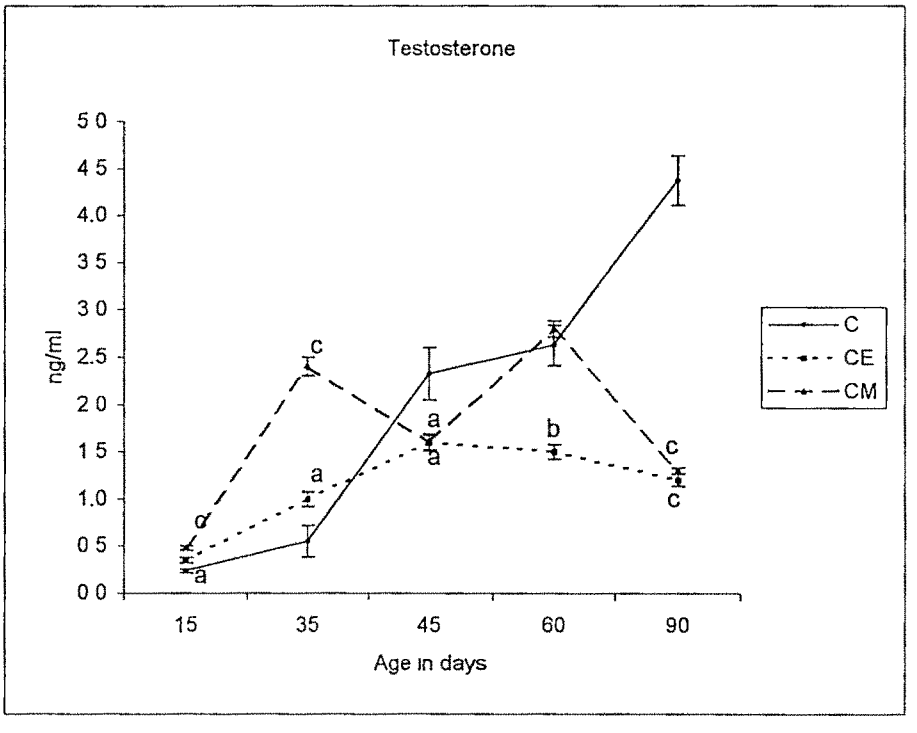
Fig.5b

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

C - Control, **CM+MT** - High Dose Corticosterone evening injection+Evening Melatonin injection, Values expressed as Mean ± SEM of four samples

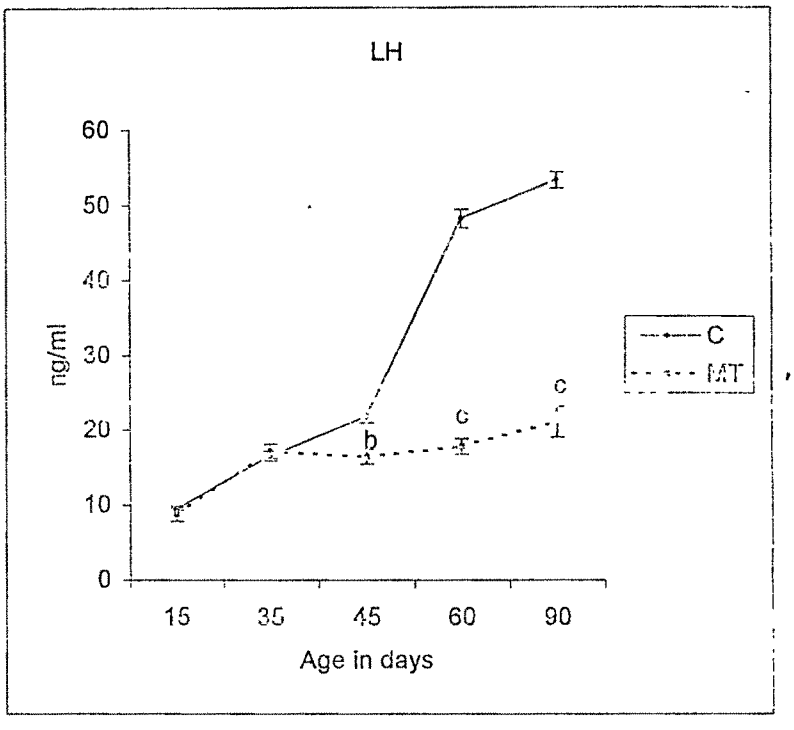
[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

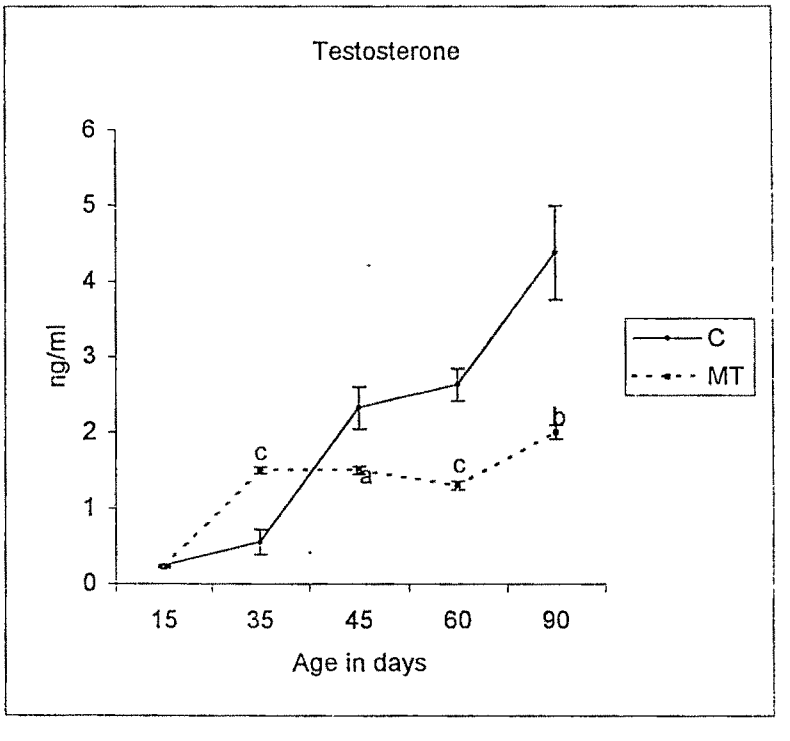




Serum LH and T levels (ng/ml) in Control and Corticosterone treated rats

C - Control, **CE** - High dose evening Corticosterone injection, **CM** - High 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
CM+MT	4.8^{a} ±0.48	6.15 ±0.51	9.8^{a} ±0.95	7.6 ±0.84	8.1^{b} ±0.65	0.53^{c} ±0.036	2.25^{c} ±0.058	2.53^{c} ±0.051	1.68^{c} ±0.036	1.74^{a} ±0.04	0.38^{a} ±0 029	0.40 ±0.031	0 31 ±0.025	0.42^{a} ±0.023	0.37^{b} ±0.015
* 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
* CM	3.18 ±0.05	6.90 ±0.10	5.90^{c} ±0.09	7.1^{a} ±0.12	7.5^{c} ±0.18	0.47^{a} ±0.06	1.4^{c} ±0.04	1.18 ±0.09	1.73^{c} ±0.069	2.48 ±0.094	0.42^{b} ±0.021	1.6^{c} ±0.038	0.7^{b} ±0.017	0.8^{a} ±0.021	0.83^{a} ±0.043

C – Control, **CM+MT** - High Dose Corticosterone evening injection + Evening Melatonin injection, **CM** - High 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 3

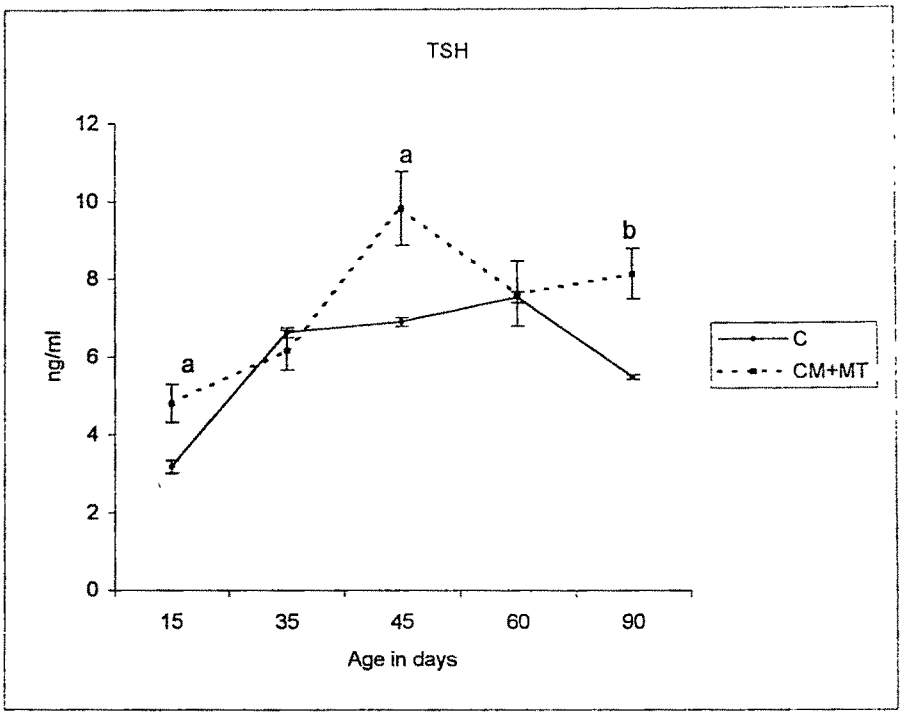
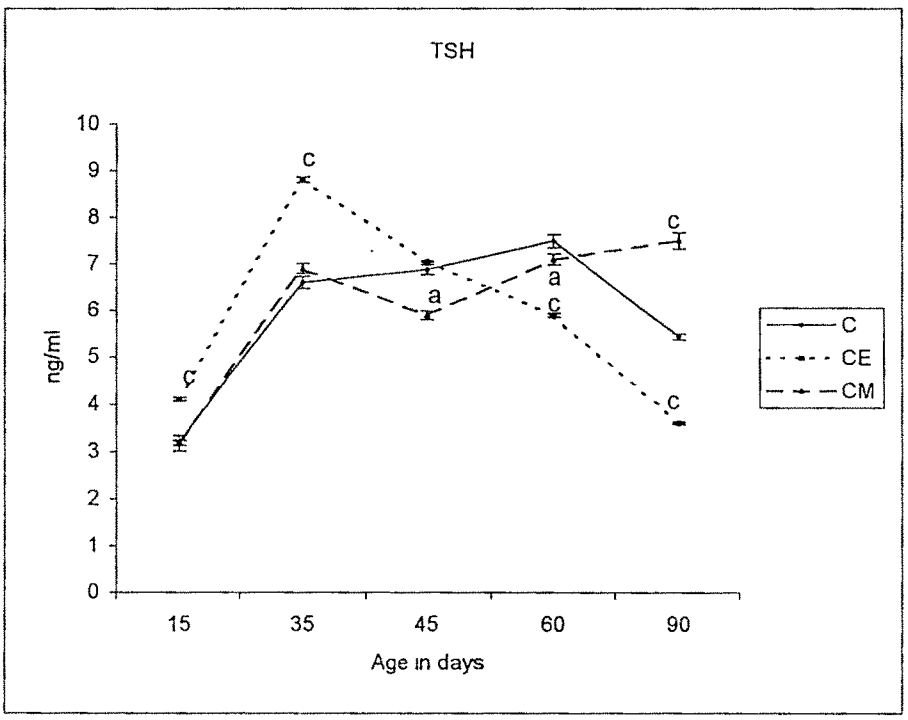
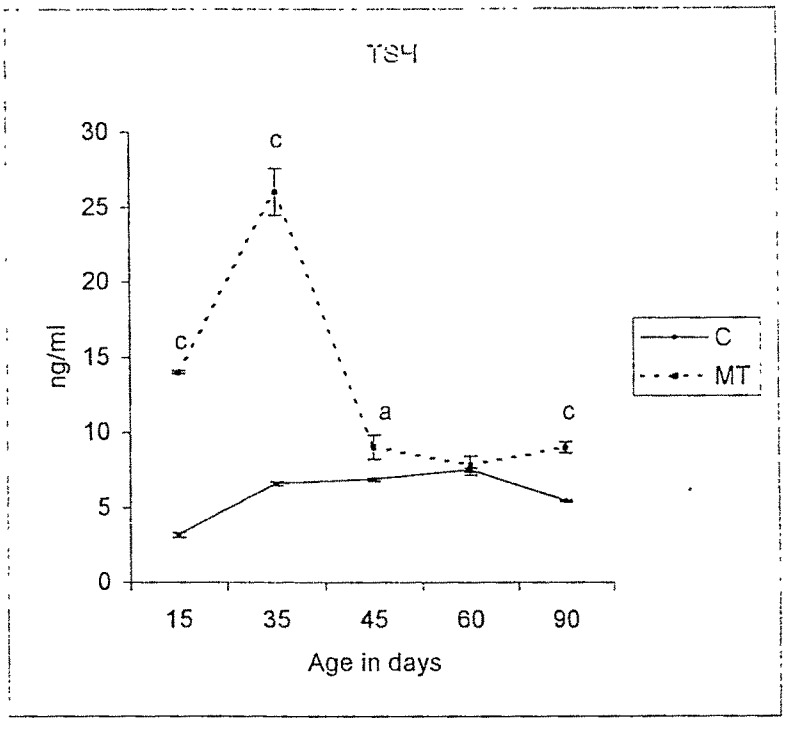


Fig.6: Serum TSH level (ng/ml) in Control and Corticosterone + Melatonin treated rats

C - Control, **CM+MT** - High 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 TSH level (ng/ml) in Control and Corticosterone treated rats
C - Control, CE - High dose evening Corticosterone injection, CM - High dose morning Corticosterone injection, Values expressed as Mean ± SEM of four samples [a] -p< 0.05, [b] -p< 0 005, [c] -p< 0.0005

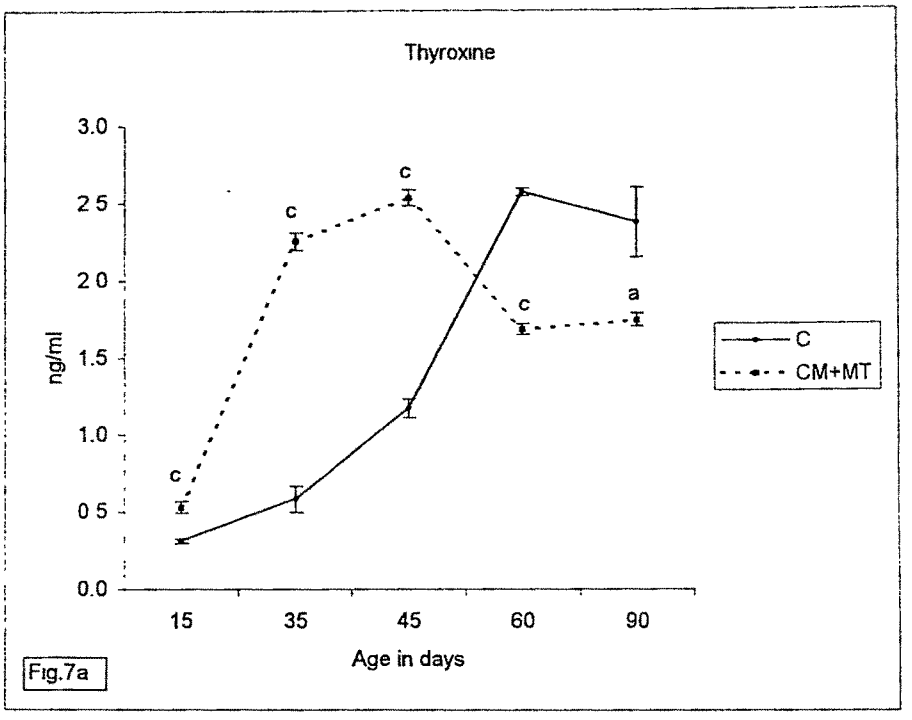


Serum TSH level (ng/ml) in Control and Melatonin treated ra

C – Control, MT – Melatonin treated

Values expressed as Mean ± SEM of four samples

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



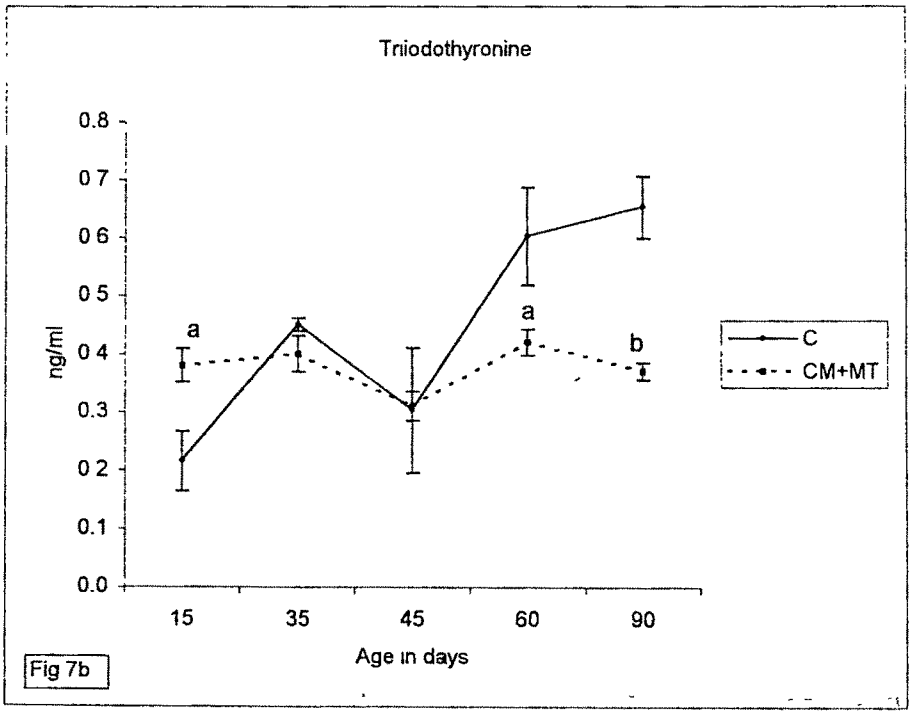
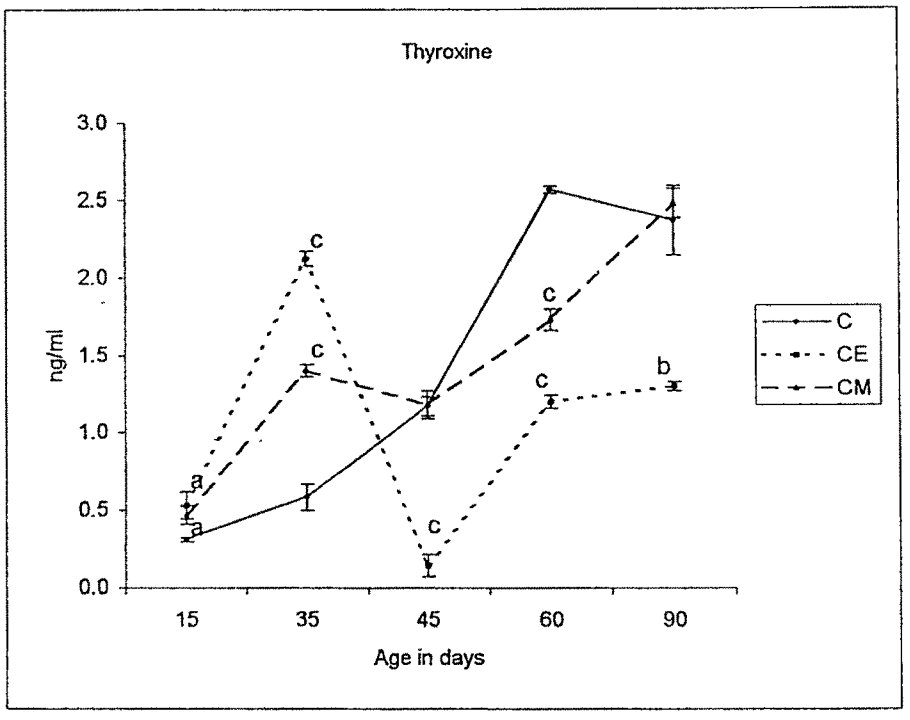
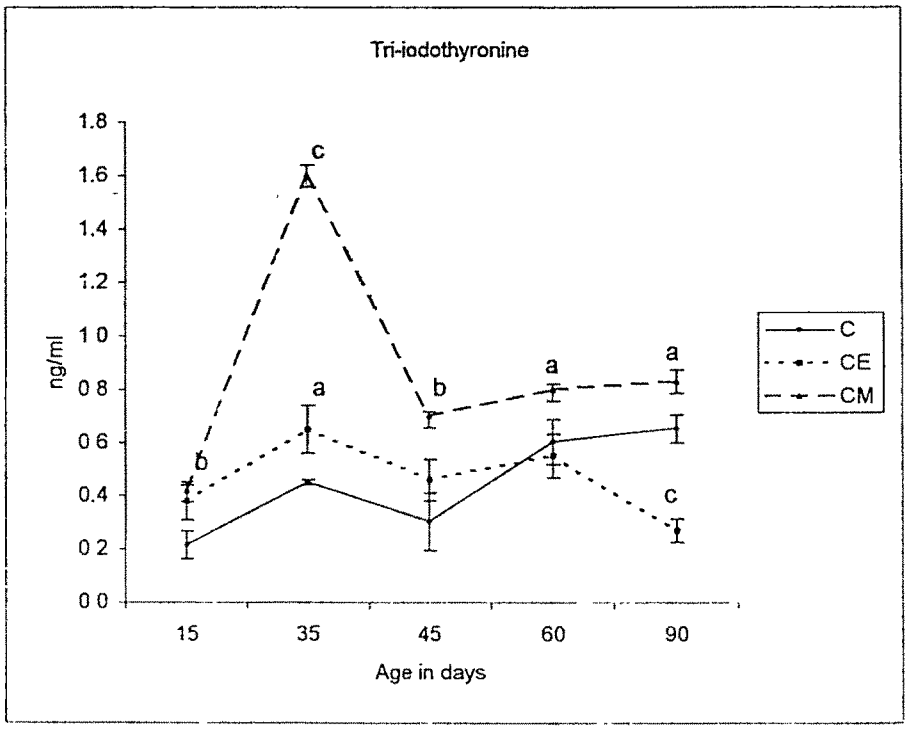


Fig.7a and 7b: Serum T_4 and T_3 levels (ng/ml) in Control and Corticosterone + Melatonin treated rats

C - Control, **CM+MT** - High Dose Corticosterone evening injection+Evening Melatonin injection, Values expressed as Mean ± SEM of four samples

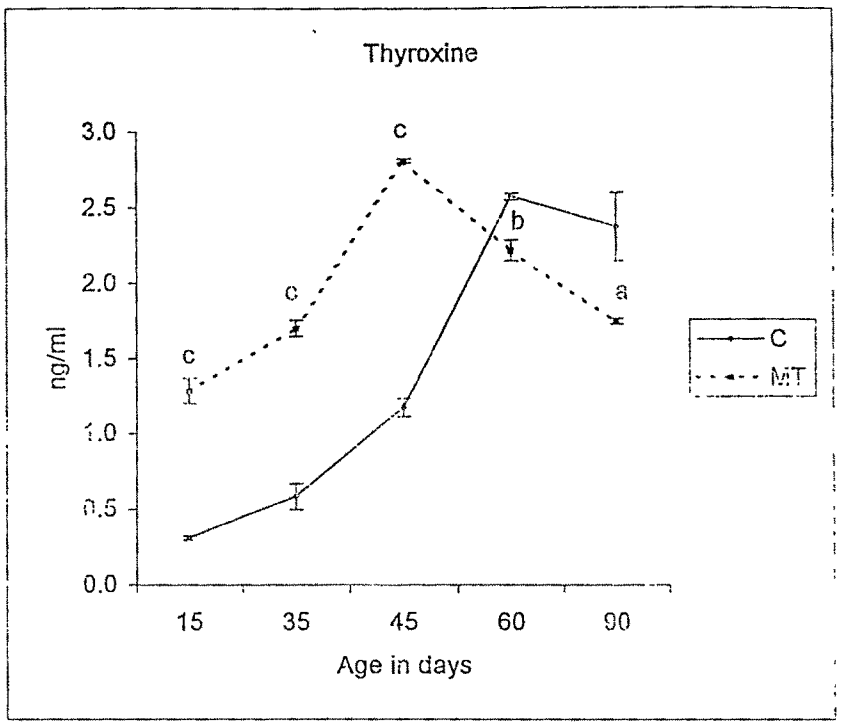
[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$





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

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



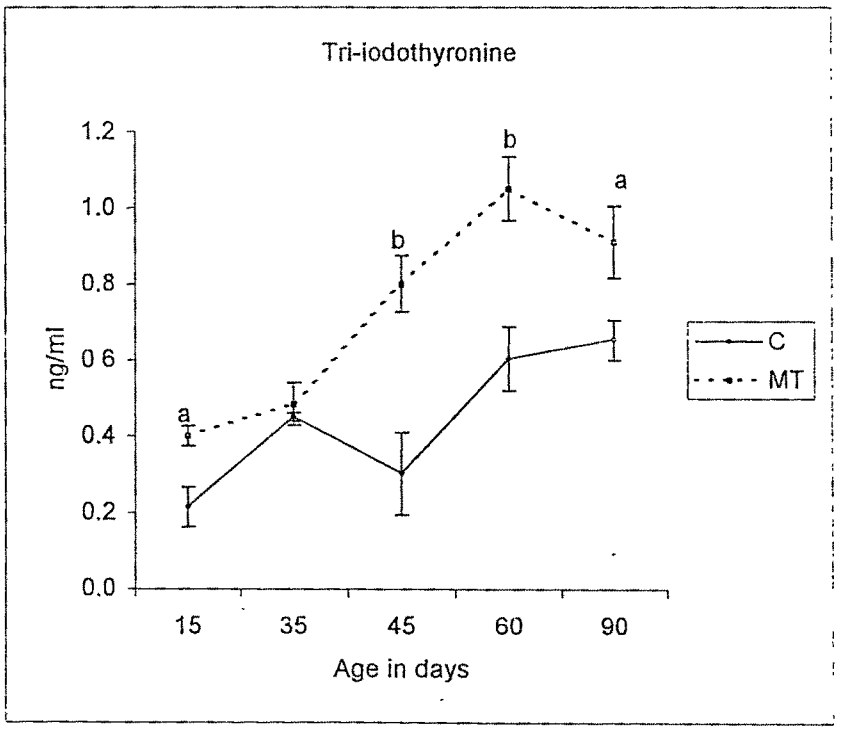


Fig.7a and 7b: Serum T_4 and T_3 levels (ng/ml) in Control and Melatonin treated rats.

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

Values expressed as Mean ± SEM of four samples

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

LH and Testosterone:

The LH level increased continuously from 15 to 60 days but was significantly lower than the age matched controls (Table 4; Figure 5a). The serum testosterone titre also showed a continuous increase from 15 to 90 days like in controls but, at 15 and 35 days the levels were significantly greater than in controls and, at 45, 60 and 90 days, significantly lesser (Table 4; Figure 5b).

DISCUSSION:

A previous study involving concurrent corticosterone and melatonin administration in the evening in rat neonates from day 0 to day 21 had reported deleterious effect on spermatogenesis without altering testes weight and, altered neuroendocrine axes of thyroid, adrenal and gonad. The present study involving administration of corticosterone in the morning and melatonin in the evening has recorded pronounced deleterious effects on spermatogenesis and testicular histoarchitecture with unique changes in hormone profiles suggesting time specific influence of corticosterone-melatonin combination. Previous investigations also showed that morning administration of corticosterone has favourable influence on timing of puberty and spermatogenesis, but a doubled dose of corticosterone has attendant deleterious effect on survival of mature germ cells. In another study, neonatal melatonin administration in the evening was shown to delay

establishment of spermatogenesis, but increase the germ cell number with a greater degree of germ cell degeneration. The present study records a potentiation of the deleterious effects of both higher morning corticosterone and evening melatonin in terms of testis functions and, also other changes which are either due to corticosterone or melatonin alone or, even a unique synergistic effect. The CM+MT animals in the present study show consistently higher weights during the treatment as well as post-treatment periods. The absolute weight of testes was also higher till 60 days but at 90 days the same was lesser than the controls. In terms of relative weight, the testes weight is lower than the controls in all time periods. The changes in body and testes weight are well reflected by the growth rates. Similar changes in body and testes weights were shown by corticosterone and MT animals (Chapters 1 & 3). Whereas the adult body weight of CM+MT rats is intermediate to that between MT and corticosterone rats, the relative testes weight was lowest amongst the three groups and more comparable to MT treated rats (Table 1).

The similarity to melatonin effect on testes weight is further emphasized by the documented degenerative and apoptotic germ cell loss together with premature whole scale denudation of the germinal epithelium on the luminal side. These changes are significantly greater than that observed in MT animals. The very poor number of sperms observable in the tubules of CM+MT animals suggests some detrimental effect on

spermiogenesis and/or their adhesional association with Sertoli cells. Previous studies on corticosterone treatment had shown higher sperm mass indicating a favourable influence of neonatal corticosterone. But clearly this favourable influence of corticosterone is completely nullified and the negative melatonin effect on spermatogenesis is highly potentiated. This effect of melatonin on corticosterone background seems to be more detrimental to the survival of spermatids and spermatozoa than that observed with a combination of evening corticosterone and melatonin. Apparently, the degree of melatonin effect in conjunction with corticosterone is time dependent with morning treatment being more harmful than evening administration. The CM+MT rats also show reduced tubular length and basement membrane area, both of which are comparable to MT treated rats (Chapter 1). Whereas the Sertoli cell number is similar as in MT rats, the germ cell number was lesser than in either C, MT or CM rats. This is reflected in the greater degree of germ cell loss (25%) (Table 3). Some explanations albeit speculative that could be suggested for the qualitatively and quantitatively deleterious effect of CM+MT combination on spermatogenesis are:

1. A relatively higher level of corticosterone during the preweanling period might exert an opposite damaging influence on germinal epithelium compared to an optimal level as seen in previous studies on exposure of neonates to corticosterone (Chapters 2 & 3).

Higher levels of corticosterone at 15 and 35 days in melatonin rats and still higher levels in CM+MT animals could possibly support the above contention.

2. It could also be due to either an up regulation of corticosterone receptors or increased sensitivity to melatonin or vice a versa on Sertoli and/or germ cells, resulting in the observed detrimental effect on spermatogenesis.

3. The levels of LH (throughout) and T (from 45 days onwards) are significantly lower in CM+MT animals, which could be another factor, which affects the spermatogenic process, especially the post meiotic stages. However, the increased T levels at 15 and 35 days could account for the hastening of spermatogenesis and appearance of sperms at 45 days. Though the reason for the observed long-term effects of neonatal morning corticosterone and evening melatonin seems to be a probable permanent genetic reprogramming of the Sertoli and germ cells, possible effects on the expression of Sertoli and germ cell adhesion molecules could be a factor of significance. In this connection, many recent reports have identified probable candidate molecules for effective germ cell-Sertoli cell adhesion (Chapter 4). The CM+MT combination also seems to have a permanent lowering effect on the set point of hypothalamo-hypophyseal-gonadal (HHG) axis, as can be deduced

from the significantly lowered LH & testosterone levels, right from 15th neonatal day. Though the CM+MT combination shows a significantly elevated corticosterone level during the treatment period (15 days) as well as the post-treatment period upto puberty (35 and 45 days), the adult level of corticosterone (60 and 90 days) was significantly lower. This indicates a lowering effect of the neonatal hormonal combination on the central set point of hypothalamo-hypophyseal-adrenal (HHA) axis. There seems to be a corticosterone effect as, the observed levels are quiet similar to those observed in our previous works on neonatal morning corticosterone excess (Chapters 2 & 3). Melatonin seems to be incapable of negating this effect of corticosterone as, MT rats did not show altered adult corticosterone levels. The hypothalamo-hypophyseal-thyroid (HHT) axis seems to be differentially affected with, TSH levels being significantly elevated throughout and, the T_4 and T_3 levels significantly lower in adult condition. It may be speculated that TRH seems to be increased while the sensitivity of thyroid to TSH is decreased on a long-term basis. These effects of CM+MT combination on the HHT axis are quite distinct from those of morning corticosterone or evening melatonin alone (Table 5).

In conclusion, it could be said that neonatal morning corticosterone-evening melatonin excess has further deleterious unfavourable influence

on adult testis functions, a potentiated influence of melatonin on a corticosterone background, even more potentiated than that found in either evening corticosterone or evening melatonin treatment 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.

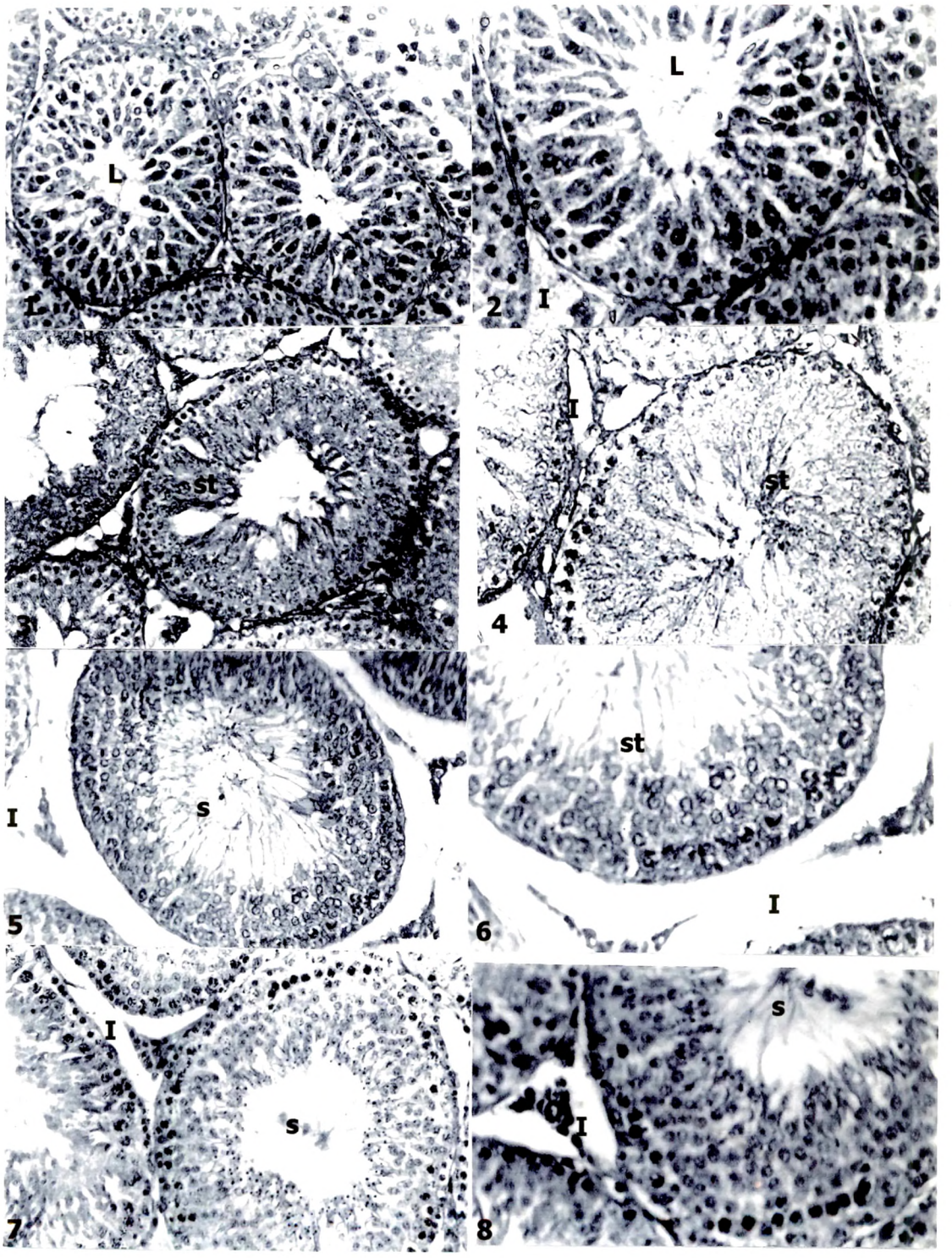

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PLATE – VIII a

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

Figures 1 to 3 : Sections of testis of 35 day old CM+MT rats showing, elongated spermatids.

Figures 4 and 5 : Section of 45 day old testis in CM+MT rats showing poor sperm density and germ cell number.

CM+MT – Morning Corticosterone + Melatonin injection

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

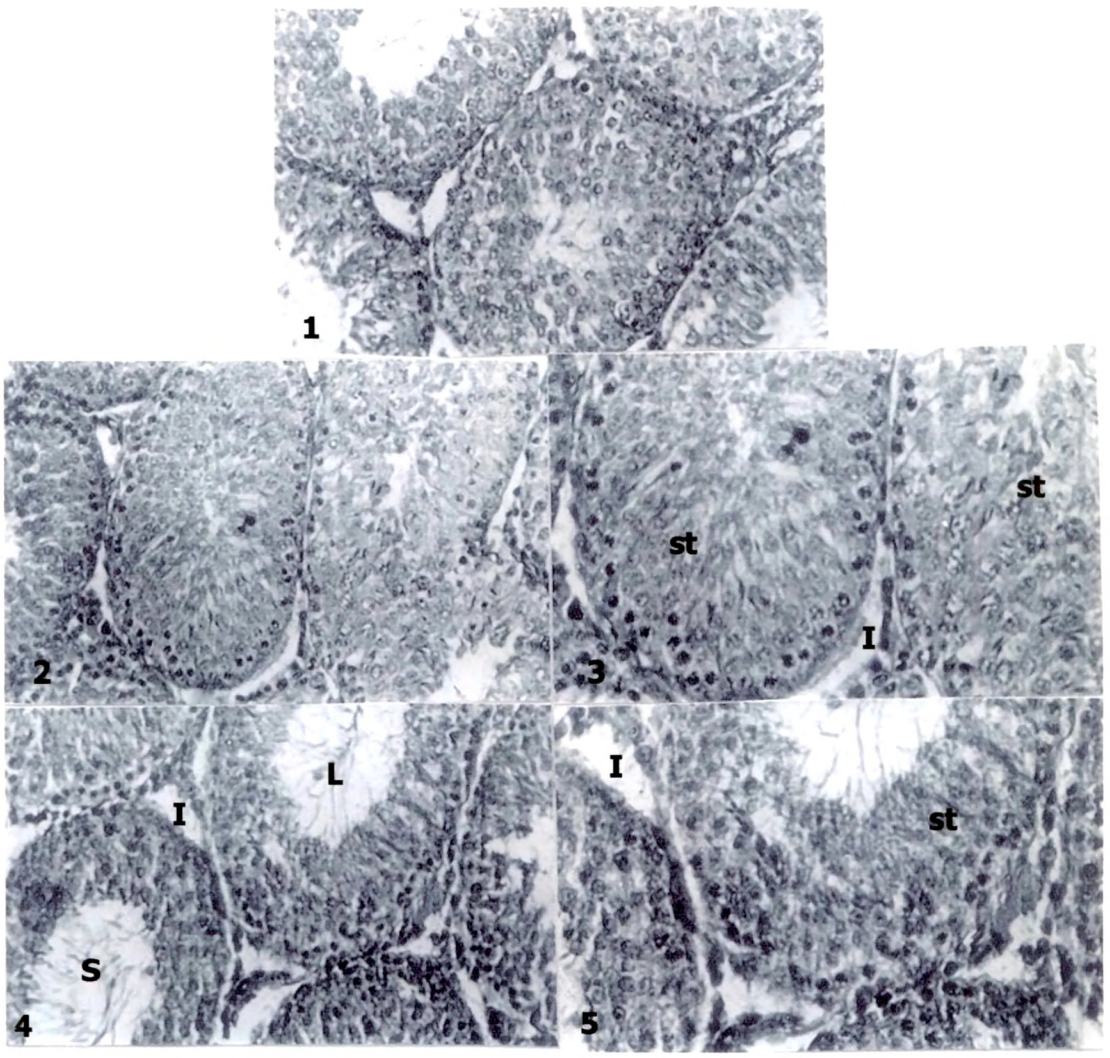
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PLATE – VIII b

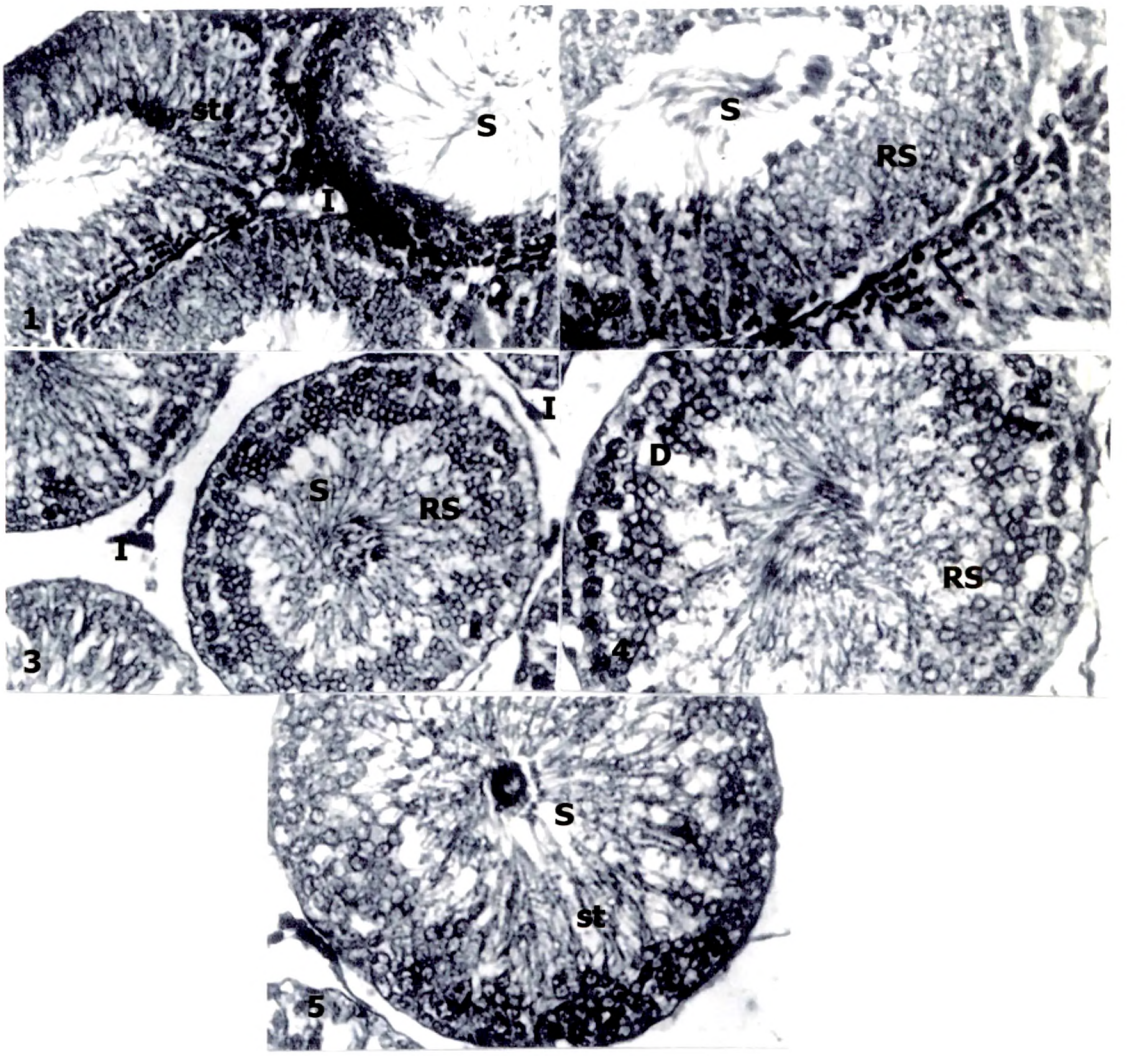
Figures 1 – 5: Photomicrographs of sections of testis in rats treated with corticosterone and melatonin.

Figures 1 and 2 : Sections of testis of 60 day old CM+MT rats showing, poor sperm density.

Figures 3 to 5 : Section of 90 day old testis in CM+MT treated rats showing, greater degenerative changes dislodgement and less number of germ cells.

CM+MT – Morning Corticosterone + Melatonin injection

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

Previous works had shown definite influence of neonatal corticosterone excess (morning or evening) or melatonin excess on adult testis structure and functions. In the present study, a combination of hypercorticalism by morning administration of corticosterone and hypermelatonemia by administration of melatonin in the evening has been induced neonatally from day 0 to day 21 and its impact has been access in terms of body and testes weight, histoarchitecture and histometry of testis and hormonal profile in the adult stage in the Charles-Foster strain of rats. Though there was no significant difference in body or absolute testes weights, the relative weight of testes was significantly lower. The seminiferous tubule diameter, germinal epithelial thickness and total germ cell count per unit length of tubule were all increased. But the total length of seminiferous tubule, total basement membrane area, Sertoli cell count and actual germ cell count were all significantly decreased. The total germ cell loss was also high. The serum corticosterone, LH and testosterone were significantly low on a long-term basis. The serum T_4 and T_3 levels were lower but the serum TSH level was higher. Most of the negative effects on testis features are similar to those seen in melatonin treated rats and, more pronounced in comparison.

Previous studies on corticosterone administration had shown increased germ cell number and sperm mass, and reduced germ cell apoptosis. However, in the present case these favorable influences of corticosterone were nullified with simultaneous melatonin administration. It is inferred that the deleterious effects of melatonin are potentiated in a higher corticosterone background. Apart from these effects on testes, neonatal morning corticosterone plus evening melatonin combination also has a permanent lowering effect on the set points of hypothalamo-hypophyseal-gonadal (HHG) and hypothalamo-hypophyseal-adrenal (HHA) axes. The hypothalamo-hypophyseal-thyroid (HHT) axis seems to be differentially affected with a higher thyrotrophin releasing hormone (TRH) secretion or thyrotrope sensitivity and lower thyroid sensitivity to TSH. Overall, it is concluded that neonatal hypercorticalism and hypermelatonemia have significant and potent deleterious effects of neonatal hormonal interactions.