CHAPTER - III

Transient neonatal exposure to glucocorticoids upto weanling hastens spermatogenesis and increases germ cell number but affects the quality of spermatogenesis: II. Dose dependent effect

Elevated levels of glucocorticoids due to stress, Cushing's syndrome or even long term exposure to exogenous glucocorticoids have been shown to disturb male gonadal functions (Mackenna *et al.,* 1979; Blair and Light, 1984; Collu *et al.,* 1984; Mac Adams *et al.,* 1986; Orr *et al.,* 1994; Monder *et al.,* 1994a; Monder *et al.,* 1994b; Maric *et al.,* 1996; Chatterton *et al.,* 1997; Harriba, 1997). Even in animals, stress induced elevation in glucocorticoids has been reported to affect many aspects of testicular function (Kumar and Rao, 1976; Collu *et al.,* 1984; Orr *et al.,* 1994; Maric *et al.,* 1996; Chatterton *et al.,* 1997;). Foetal and neonatal periods of development do represent critical phases and any alteration in the internal *milieu* at these stages is likely to have subtle or profound effects. In this respect hormonal alterations in the foetal environment have been reported to influence the adult phenotype (Barker, 1999). Though glucocorticoids are regarded essential for the maturation of foetal organ systems (Baxter and Rousseau, 1979), the exposure of foetuses to excess glucocorticoid has been shown to retard

growth and precipitate diseases in adult (Benedicktsson *et al.,* 1993; Levitt *et al.,* 1996; Lindsay *et al.,* 1996). This has led to the premise that glucocorticoids are involved in the programming of postnatal development of various systems. In relation to reproductive functions, foetal exposure to glucocorticoids has been reported to be detrimental for postnatal reproductive development resulting in delayed onset of puberty in the female off springs of mothers subjected to stress (Politch and Herrenkohl, 1984a) or treated with adreno-corticotrophic hormone (ACTH) during gestation (Harvey and Chevins, 1997). In a recent study, Smith and Waddell (2000) had recorded reduced offspring birth weight and delayed onset of puberty in male offsprings of mothers subjected to glucocorticoid excess from gestation day 13 to term. This had made them to suggest that foetal exposure to glucocorticoid is an important determinant in the timing of puberty onset.

These observations on foetal exposure to glucocorticoids gave impetus to study the possible influence of glucocorticoid excess during the postnatal period on the development of the adult reproductive system. To test the hypothesis that glucocorticoids in preweanling postnatal period could influence the functional maturation of testes and puberty onset, a study was conducted earlier on the influence of time dependent exposure of rat neonates from day 0 to day 21 on prepubertal and postpubertal testis functions (Chapter 2). The above study revealed earlier puberty onset and increased adult testis size with higher germ cell number and sperm density,

more favourable with evening schedule. Since the above study involved evaluations of the effect of mild glucocorticoid excess, the present study is planned to assess the impact of a doubled dose of corticosterone. Puberty onset and qualitative and quantitative aspects of spermatogenic process and alterations in the hormonal profiles of corticosterone (CORT), thyroid stimulating hormone (TSH), tri-iodothyronine (T$_3$), thyroxine (T$_4$), luteinizing hormone (LH) and testosterone (T) have been evaluated.

MATERIALS AND METHODS:

Animals and Maintenance:

As in chapter one.

Preparation of Corticosterone:

As in chapter two.

Experimental Protocol:

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

<u>Group I (control) (C):</u>

Newborn rat pups maintained till 90 days served as controls. This
consisted of 3 subgroups (as follows) of 30 animals each:

(i)   Control rats **(N)**

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

(iii)   Injected *i.p.* with vehicle (0.9% saline) in evening (1600 hrs) **(C$_E$)**

<u>Group II (Corticosterone treated) (CORT):</u>

Newborn rat pups were injected *i.p.* with Corticosterone in the following
doses:

Corticosterone high dose:

    Day 0 to 10   → 2μg CORT/ animal/day

    Day 11 to 21   → 4μg CORT/ animal/day

The corticosterone treated animals were divided into the following subgroups
each consisting of 30 animals:

    i.   CORT high dose in morning (0800 hrs) **(CM)**

    ii.   CORT high dose in evening (1600 hrs) **(CE)**

<u>Parameters and Methods of Evaluation:</u>

    As in chapter one.

<u>Histology and Histometry:</u>

    As in chapter one.

Hormone Assays:

As in chapter one.

Statistical Analysis:

As in chapter one.

RESULTS:

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

Postnatal Growth:

The experimental animals showed higher body weight during the treatment period, which persisted even in the post-treatment periods extending upto 60 days (Table 1; Figure 1a). However the final weight at 90 days was marginally higher in CE and significantly lower in CM rats compared to controls. The body growth rate was also similarly higher upto 60 days and lower between 60 and 90 days in the experimental groups as compared to controls (Table 2; Figure 3a). The absolute weight of testes was higher in the post-treatment period upto 60 days. However, the final weight at 90 days was the same as that of the control in CM but, higher in CE. In contrast, the relative weight of testes of experimental animals remained consistently lower than that of control till 60 days but at 90 days, the relative

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Table 1: Chronological alterations in body weight (g) and absolute weight (g) and relative weight (g/100 g) of testes in Control and Corticosterone 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	29.98 ±1.645	94.16 ±3.804	129.69 ±3.667	206.4 ±6.396	336.15 ±6.280	0.291 ±0.015	0.956 ±0.078	1.48 ±0.58	2.52 ±0.85	3.17 ±0.104	0.986 ±0.056	1.24 ±0.064	1.158 ±0.083	1.34 ±0.067	0.934 ±0.046
CE	34.8[a] ±1.789	115.3[b] ±1.352	162.3[c] ±2.552	270.3[c] ±4.279	350.00 ±7.785	0.147 ±0.010	0.863 ±0.44	1.72 ±0.74	2.933 ±0.042	3.51[a] ±0.013	0.402[c] ±0.021	0.784[c] ±0.042	1.085 ±0.081	1.071[b] ±0.034	1.035 ±0.03
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, CE – High Dose Corticosterone evening injection, CM – High Dose corticosterone morning injection

Values expressed as Mean ± SEM of six animals. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$
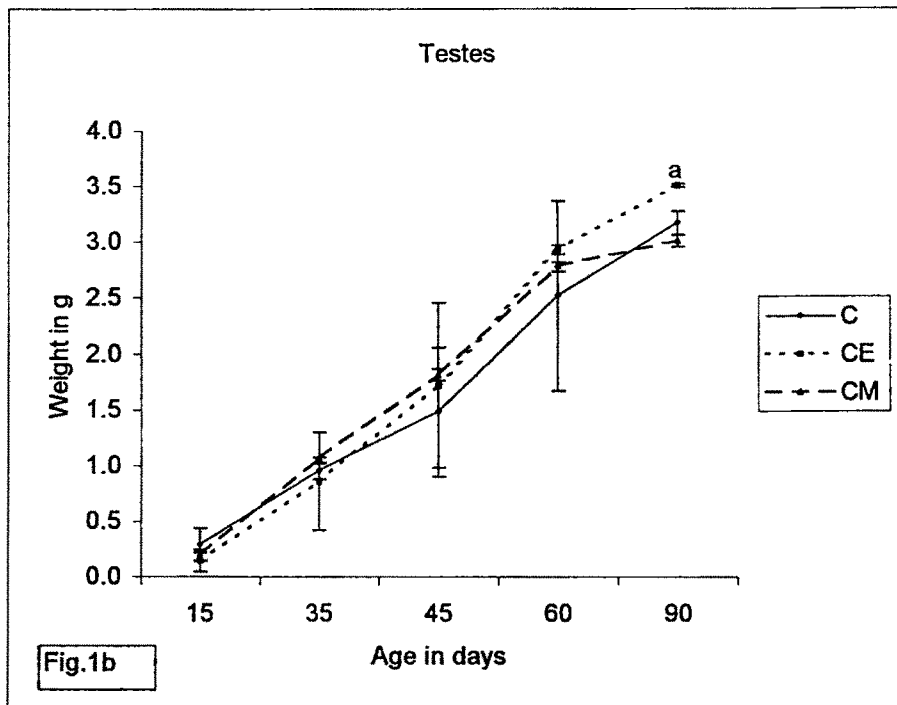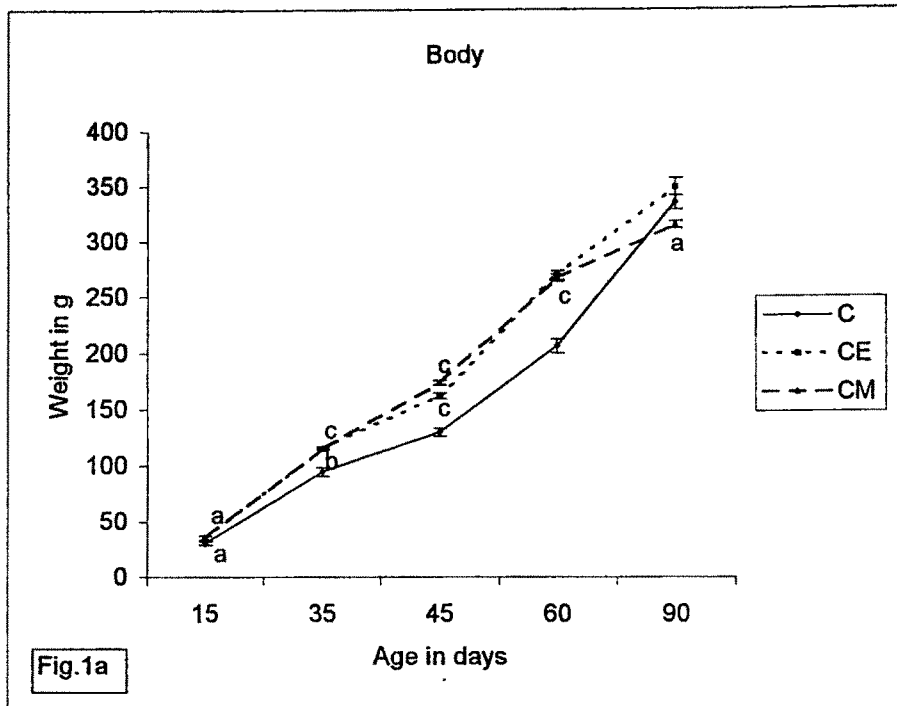
Fig. 1a and 1b: Chronological alterations in body weight (g) and absolute
weight (g) of testes in Control and Corticosterone treated rats.
C - Control, CE - High dose evening Corticosterone injection, CM - High dose
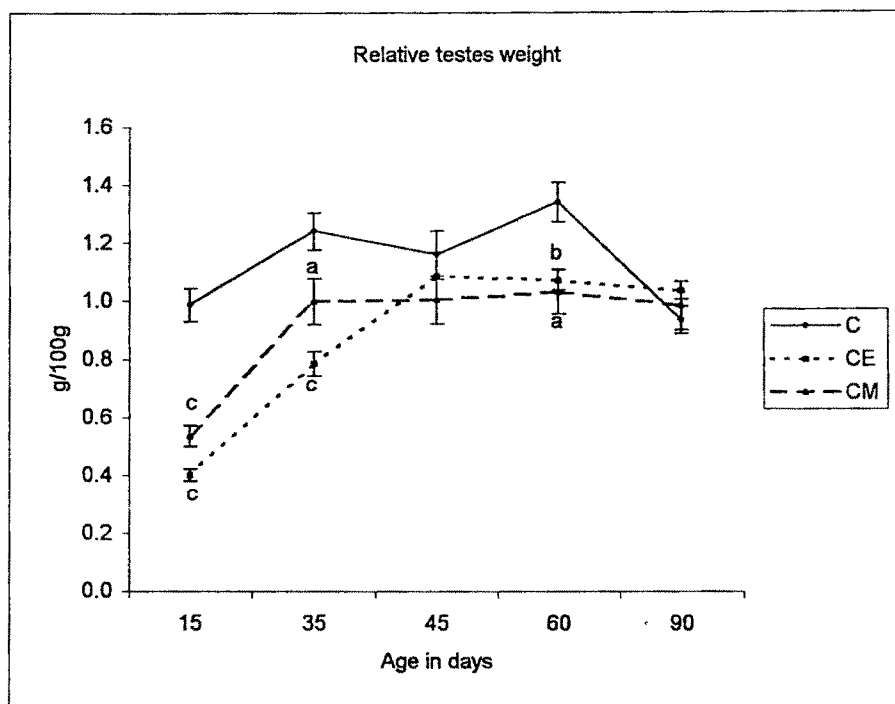morning Corticosterone injection, Values expressed as Mean ± SEM
of six animals [B89]

Fig.2: 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 $p< 0.0005$

Table 2: Per day Body and Testes Growth Rate (g/day) in Control and Corticosterone 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	1.627	3.209	3.55	5.114	4.325	0.0194	0.033	0.052	0.069	0.022
CE	1.92	4.023	4.703	7.2	2.655	0.009	0.036	0.086	0.081	0.019
CM	1.953	4.000	5.81	6.28	1.601	0.013	0.044	0.074	0.065	0.0073

C – Control, **CE** - High Dose Corticosterone evening injection, **CM** - High Dose corticosterone morning injection
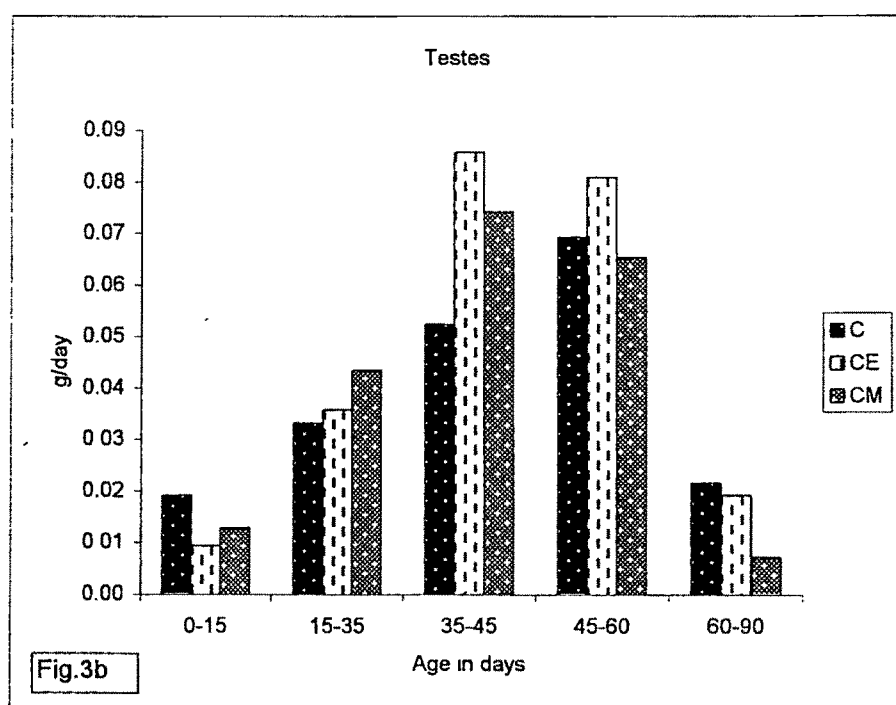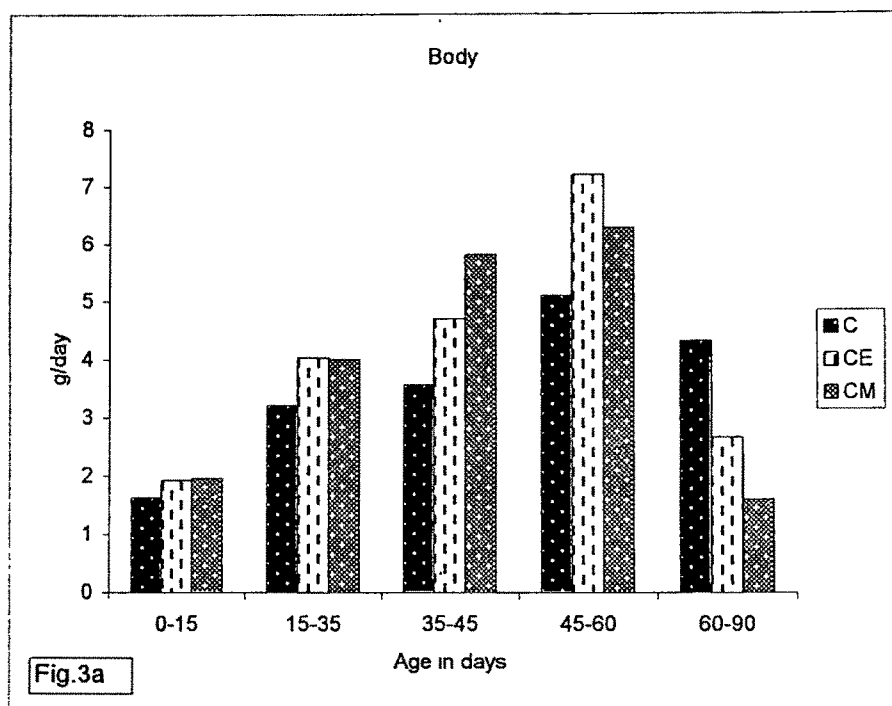
Fig. 3a and 3b: Per day Body and Testes growth rate (g/day) in Control and Corticosterone treated animals

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

weight of experimental animals was higher than controls (Table 1; Figure 2). The growth rate of testes in CM animals was higher than the controls upto 45 days and almost similar to controls between 45 and 60 days, followed by a sharp decline between 60 and 90 days (Table 2; Figure 3b). In the case of CE animals, the growth rate of testes was significantly higher than that of the controls between 35 and 60 days followed by a decline between 60 and 90 days.

Histology and Histometry:

Testicular and tubular volumes were slightly more in CE animals and less in CM animals. The testis sections of control animals depicted full spermatogenic activity and appearance of sperms in the tubules at 60 days. In contrast in CM and CE animals, spermatogenesis was fully established by 45 days marked by the appearance of sperms. Though there was no difference in the ultimate length of the tubules attained at 90 days in either of the two experimental groups, it was slightly more than the control length. The total basement area was slightly more in CE animals and slightly less in CM animals as compared to 90-day controls. Most of the tubular growth occurred after 35 days in CE animals like in controls but, in CM animals, the same occurred by 35 days. The testis of experimental animals at 60 and 90 days depicted increased germ cell number. This was paralleled by increased diameter of tubules and thickness of germinal epithelium. However, there

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Table 3: Histometric enumeration of seminiferous tubules of Control 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 cm²	SC_N x10⁶	TGC_T x10⁶	AGC_T x10⁶	TGC_M x10⁶	AGC_M x10⁶	% 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	1.602 ±0.080	0.0309 ±0.0001	0.0082 ±0.0009	1.522 ±0.040	2480.981 ±89.600	217.864 ±2.260	34.73 ±1.900	352.0[c] ±5.900	327.35[c] ±10.360	14.110 ±0.369	13.19 ±0.680	7.040 ±1.780
CM	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

C – Control, CE – High Dose Corticosterone evening injection, CM – High Dose Corticosterone morning injection

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

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.

appeared to be increased tendency for sloughing off of advanced stages of germ cells like spermatids and spermatozoa. The Sertoli cell count was marginally higher in CM testis but the total number of germ cells (theoretical as well as actual) was not different from the controls. In CE testis the theoretical germ cell count was higher by 13% while the actual count was still higher by 17%. The observed germ cell degeneration was higher in CM, similar to controls and, lesser in CE (Table 3) (Plates I, Va, Vb, VIa, VIb and VIc).

Serum Hormone Profile:

Corticosterone:

Serum corticosterone levels were elevated in CM and CE animals during the treatment period as well as post-treatment periods lasting upto 45 days. Thereafter, though the control animals showed significant elevation in corticosterone levels at 60 and 90 days, the level in CM and CE animals reduced from that at 45 days and remained significantly lower throughout (Table 4; Figure 4).

TSH, T_4 and T_3:

The serum TSH, T_4 and T_3 levels were significantly higher in the corticosterone treated groups during the treatment periods as well as subsequently thereafter, upto 45 days. The adult periods (60 and 90 days) were marked by significantly sub-normal TSH, T_3 and T_4 levels in CE but, in

Table 4: Serum Corticosterone, LH and T levels (ng/ml) in Control and Corticosterone 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 ±0.235	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	17.2^c ±0.786	18.2^c ±1.05	26.7^c ±0.537	14.0^c ±1.103	15.0^c ±0.689	17.81^c ±0.543	29.9^c ±1.67	18.0 ±1.82	25.02^c ±2.34	21.0^c ±2.41	0.35^a ±0.03	1.00^a ±0.08	1.600^a ±0.091	1.50^b ±0.078	1.20^c ±0.065
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, CE - High Dose Corticosterone evening injection, CM - High Dose corticosterone morning injection.

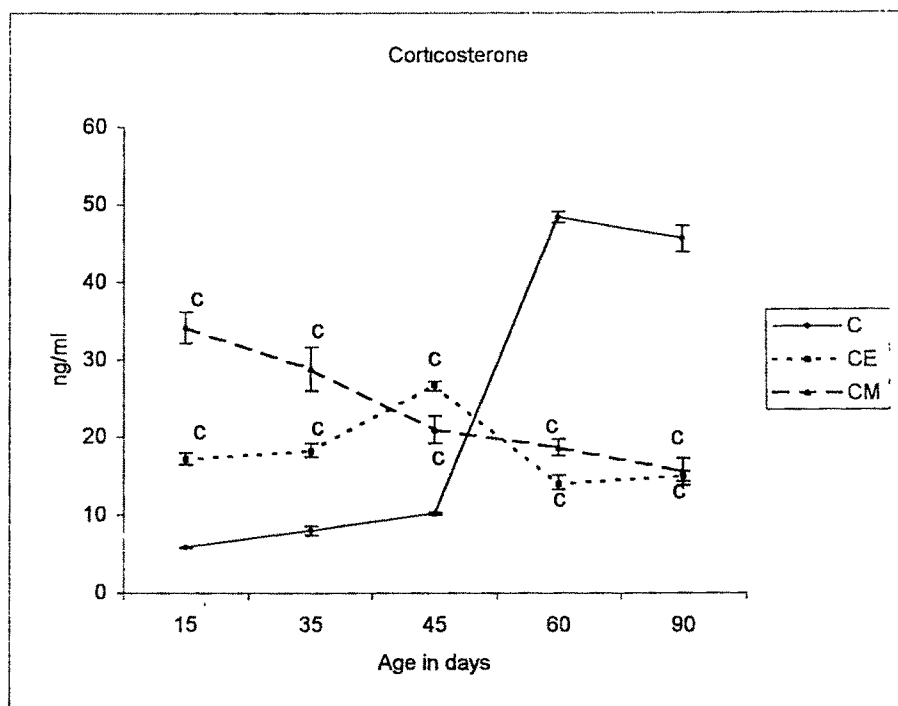Values expressed as Mean ± SEM of four samples.   [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

Fig. 4: 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
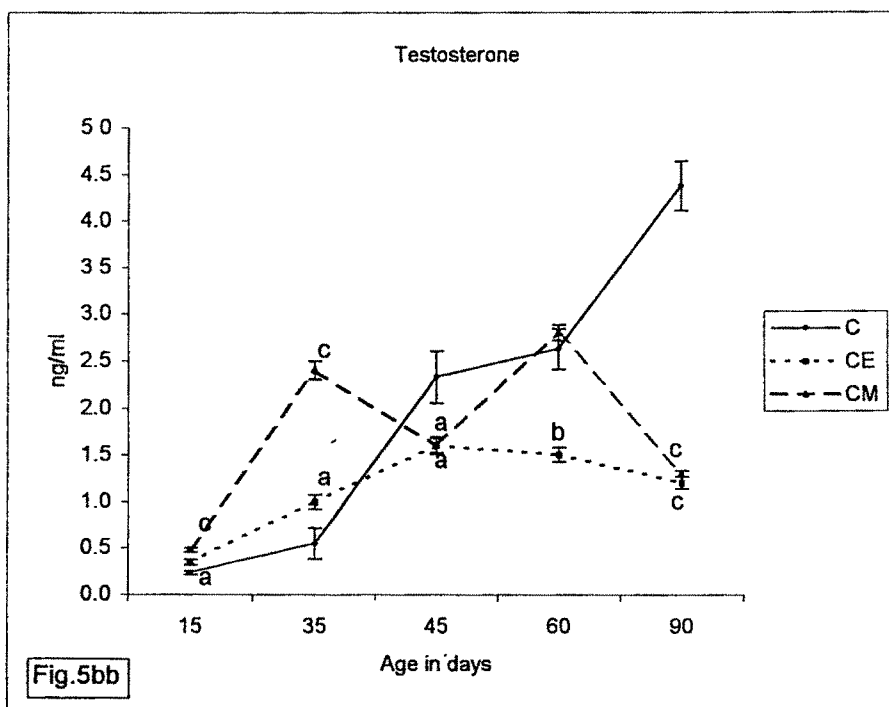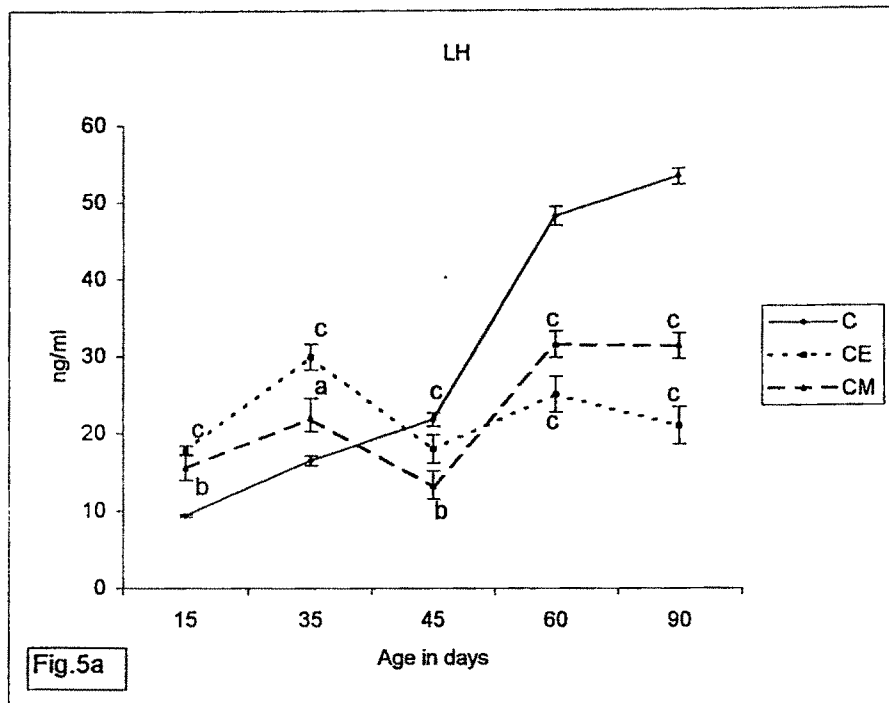of four samples [a]-$p < 0.05$, [b]-$p < 0.005$, [c]-$p < 0.0005$

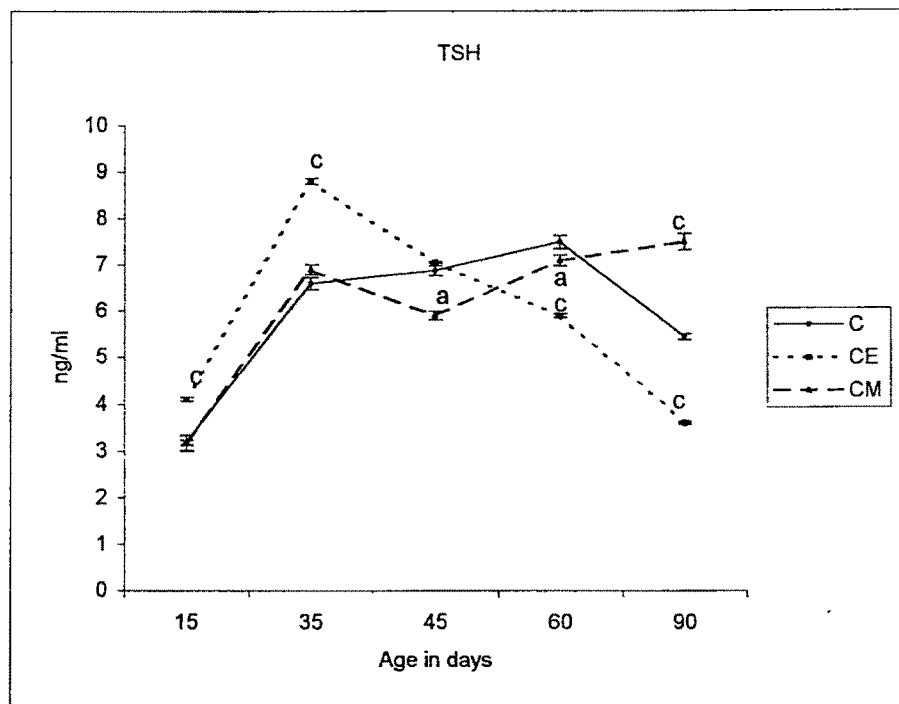Fig. 5a and 5b: Serum LH and T levels (ng/ml) in Control and Corticosterone treated rats

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

Table 5: Serum TSH, T_4 and T_3 levels (ng/ml) in Control and Corticosterone 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	4.1[c] ±0.042	8.80[c] ±0.058	7.05 ±0.021	5.90[c] ±0.041	3.60[c] ±0.029	0.53[a] ±0.087	2.12[c] ±0.048	1.43[c] ±0.071	1.20[c] ±0.043	1.30[b] ±0.03	0.38 ±0.071	0.65[a] ±0.089	0.46 ±0.078	0.55 ±0.081	0.27[c] ±0.043
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, CE - High Dose Corticosterone evening injection, CM - High Dose corticosterone morning injection.

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

Fig. 6: 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
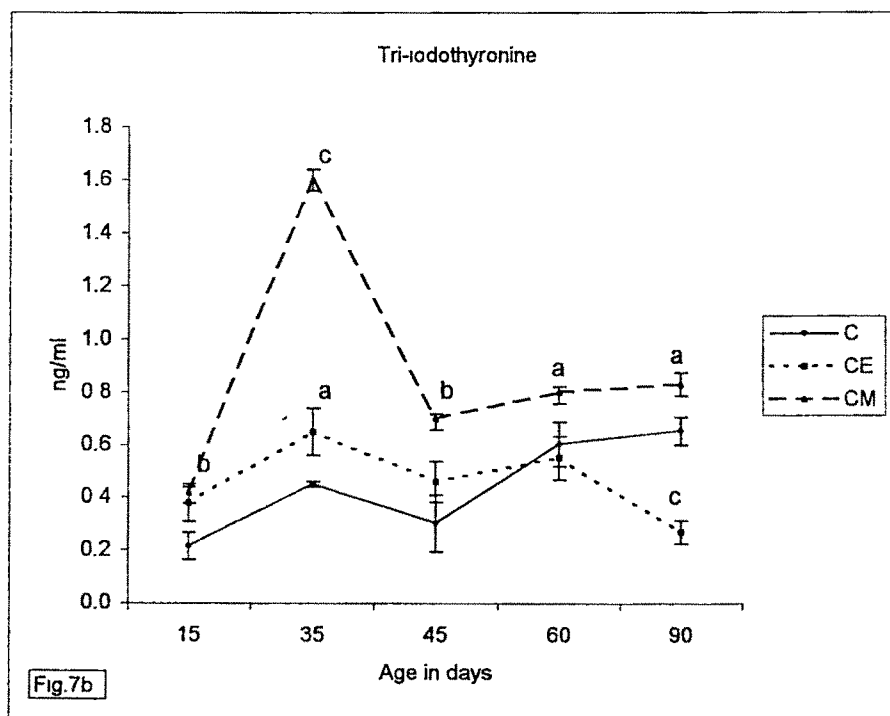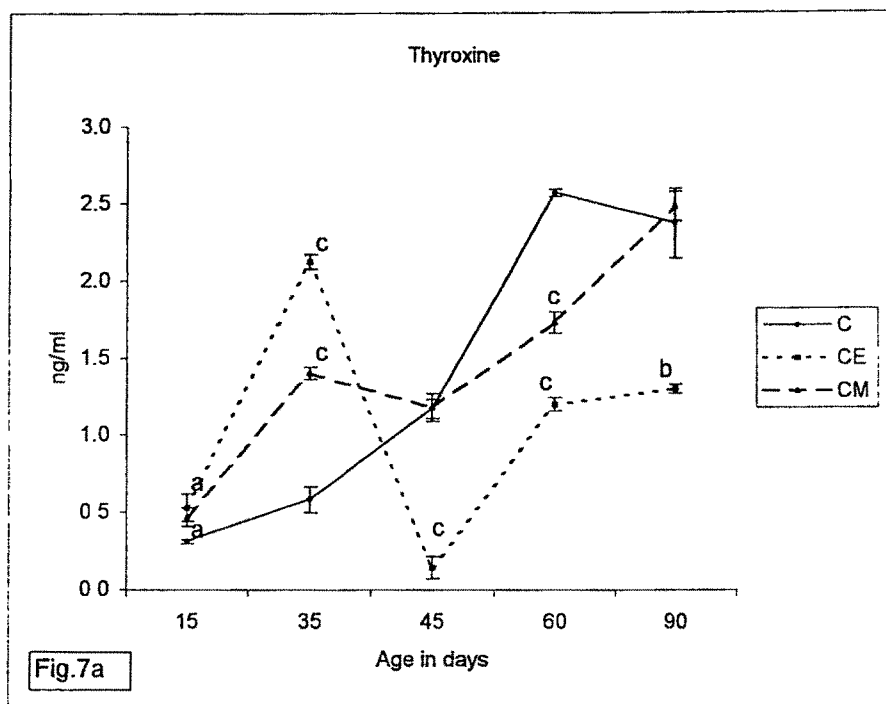of four samples [a]-$p < 0.05$, [b]-$p < 0.005$, [c]-$p < 0.0005$

Fig. 7a and 7b: 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

the CM animals, the levels were significantly higher than the controls at 90

days (Table 5; Figures 6, 7a & 7b).

LH and Testosterone:

The treatment and post-treatment periods upto 35 days were marked

by elevated serum T and LH levels in both the experimental group of animals.

Thereafter at 45, 60 and 90 days the level of both the hormones was

significantly lower than the controls (Table 4; Figures 5a & 5b).

DISCUSSION:

The previous investigation employing a low dose of corticosterone

revealed that transient postnatal glucocorticoid excess during the preweanling

period has a favourable influence on subsequent timing of puberty and on

the spermatogenic process in the adult condition. This was the first ever

report indicating a long-term effect of neonatal glucocorticoid excess in the

infant period on adult testis functions. The present investigation further

demonstrates the favourable influence of neonatal glucocorticoid excess on

early timing of puberty and spermatogenesis. However, this study involving a

doubled dose of corticosterone demonstrates a deleterious effect on the

survival of mature germ cell types suggesting a dose dependent effect of

corticosterone in the neonatal period. The favourable influence of

corticosterone is reflected in the increased body and testes weights in the

reversal period. Though the weights at 90 days, except for the testes weight

of CE animals (significantly high), were similar in the controls and experimentals; the experimentals maintained significantly higher body and testes weights till 60 days (Table 1; Figures 1a & 1b). The testes weight expressed as relative weight has also showed a similar pattern. The increased body and testes weights are indicative of faster growth and early attainment of adult size due to neonatal corticosterone excess, which is well reflected in the hastened growth rate (Table 2; Figures 3a & 3b). These observations contrast with those of other workers (Benedicktsson *et al.,* 1993; Burton and Waddell, 1994; Lindsay *et al.,* 1996; Smith and Waddell, 2000) showing decreased birth weight and delayed onset of puberty with glucocorticoid excess in the foetal period. This obviously underscores a differential developmental stage specific influence of corticosterone excess. A comparison of weight of body and testes and their growth rates between low dose of corticosterone (Chapter 2) and a higher dose (present study), emphasizes a favourable influence of corticosterone on these aspects as, the higher dose promoted faster body and testes growth towards adult weights. The dose dependent influence of corticosterone during the neonatal period is underscored by the slightly increased growth of the body during the treatment period as against a dampened growth with the lower dose (Chapter 2). Nevertheless, the final weight at 90 days was similar in both the treatment regimens due to the fall in growth between 60 and 90 days, being more precipitous in the higher dose regimen. In keeping with this, the

attainment of puberty was also enhanced in CM and CE animals by 8 days as noted by perputial separation (42 days in CM and CE versus 50 days in control, as in chapter 2).

The favourable influence of neonatal corticosterone on growth of testis is reflected in the early functional maturation of the testis marked by onset of spermatogenesis and appearance of sperms by 45 days, aspects that occur later in the control. An interesting difference was that while CM tubules showed hastened growth as, 61% of the length was attained by 35 days itself, the CE tubule length at 35 days was only 35% of the total at 90 days, just as in the case of controls (Table 3). Apart from earlier onset of spermatogenesis, the testis of corticosterone treated rats showed markedly higher density of germ cells at all ages of study. Though there is no significant difference between the theoretical increase and actual increase in the number of germ cells in CE testis, the actual increase was 17% as against 13% increase in theoretical number. The minimal difference of only 7% between theoretical and actual number suggests reduced germ cell loss in CE. This is however comparable to the loss seen earlier with lesser dose of corticosterone in the morning, but significantly more than that seen in low dose evening rats. There is relatively greater germ cell loss in CM animals and the total germ cell number was similar to the controls. Though these features are similar to what was observed with a low dosage (Chapter 2), a noticeable difference is the premature sloughing off of advanced germ cells,

especially spermatids and spermatozoa. This appeared to be more pronounced in the evening schedule indicating a time dependent influence.

A plausible explanation for the observed effects on testes may become meaningful when seen in the background of the recorded serum hormone profiles. It is clear that exposure of preweanling neonates to corticosterone has attendant effects on various hormonal axes. The higher dose of corticosterone also exerts a long-term influence by lowering the set point of hypothalamo-hypophyseal-adrenal (HHA) axis, denoted by the significantly lower levels of corticosterone in the adult (60 and 90 days) as reported for a lower dose previously (Chapter 2). Like in the previous study involving lower dose regimen, the basal corticosterone levels remain elevated beyond the exposure period during the prepubertal and pubertal periods (35 and 45 days), again signifying the possible influence of chronic neonatal corticosterone in decreasing metabolic clearance rate, as inferred earlier. The evening schedule of corticosterone decreases the hypothalamo-hypophyseal-thyroid (HHT) set point on a long-term basis while, the morning schedule increases the set point. Our previous study with lower dose of corticosterone had revealed a similar influence of elevating the set point of HHT axis. These observations taken together suggest manifestation of time dependent effect on HHT axis with high dose of neonatal corticosterone exposure.

The recorded levels of LH and T signify an increased output during the treatment and post-treatment periods (15 and 35 days) but, followed by significantly lower levels at all ages thereafter (Table 4; Figures 5a & 5b). Obviously, neonatal corticosterone exposure has a long-term permanent influence on the hypothalamo-hypophyseal-gonad (HHG) axis as well, in the form of a lowered set point. This effect seems to be independent of dose and time of exposure to corticosterone. Reprogramming of hormonal axis by neonatal glucocorticoid exposure has also been shown to affect the HHG axis (Smith and Waddell, 2000) and the HHA axis (Meaney *et al.,* 1991). The presence of glucocorticoid receptors has been shown in the neural tissue (Regan and Mckewen 1997; Daikoku and Koide, 1998), pituitary gonadotrophs (Kilen *et al.,* 1996), the ovary (Micheal *et al.,* 1993) and testis (Monder *et al.,* 1994) and can therefore account for the observed effects of neonatal corticosterone on the various hormonal axes.

The earlier onset of spermatogenesis and increased germ cell population as seen in the prepubertal and pubertal stages are possibly due to a favourable hormonal *milieu* involving higher levels of thyroid, adrenal and gonadal hormones. Whereas the elevated thyroid hormone could induce earlier Sertoli cell differentiation (Cooke *et al.,* 1991; Cooke *et al.,* 1992; Meisami *et al.,* 1992; Van Haaster *et al.,* 1992; Hess *et al.,* 1993; Van Haaster *et al.,* 1993; Cooke *et al.,* 1994;), the increased T level favours spermatogenesis (Kerr *et al.,* 1993a, 1993b; Mckinnell, 1995) and higher

corticosterone level decreases germ cell apoptosis as inferred previously (Chapter 2). The histological observation of minimal germ cell degeneration and appearance of sperm at 45 days along with increased tubular diameter, germinal epithelial thickness and ratio of tubular to germinal epithelium thickness, all attest to the above. Even the testes weight is significantly higher in CE animals and marginally higher in CM animals. The present results tend to emphasize our previous inference that neonatal corticosterone exposure can somehow attenuate the rate of normal germ cell apoptosis (Cox, 1995; Messmer *et al.,* 1999), a normal process of germ cell loss during spermatogenesis (Huckins, 1978). The decrease in germ cell apoptosis mediated by neonatal corticosterone would be by way of altered secretion of growth/paracrine factors and/or adhesion molecules from Sertoli cells by genetic re-programming. The increased germ cell number due to decreased germ cell loss is clearly reflected in the increased tubular diameter and germinal epithelial thickness with a concomitant increase in the relative weight of testes at 90 days, though more significantly in CE (Tables 1 and 3; Figure 2). The permanent reprogramming of Sertoli cells by glucocorticoid in the neonatal period could be envisaged by the non-expression of 11β-HSD (metabolising enzyme for corticosterone) in the preweanling period and the presence of corticosterone receptors in the testes (Hardy *et al.,* 1998). Though glucocorticoids have been shown to promote apoptosis in many tissues (Gonzalo *et al.,* 1993; Hassan *et al.,* 1996; Waddell *et al.,* 2000), an

anti-apoptotic influence has also been documented in some tissues (Cox, 1995; Messmer *et al.,* 1999), lending validity to the present inference.

Despite the protective action of glucocorticoids on germ cells, there is a reduced sperm density due to premature detachment and sloughing off of sperms and spermatids as revealed histologically. This might suggest that a higher dose of corticosterone might also interfere with the cytological adhesive features involving Sertoli cells and mature gem cells. The generally reported inhibitory effects of glucocorticoids on spermatogenesis and steroidogenesis could be pertinent in this context as the dosage of corticosterone generally employed in such studies is much higher than in the present one.

It can be concluded from the present observations that, glucocorticoid exposure during the preweanling neonatal period can hasten puberty, augment spermatogenesis and increase germ cell number by decreasing apoptosis on long-term basis but can interfere with the adhesive properties of mature germ cells, a feature which may be dependent on the dosage of corticosterone.
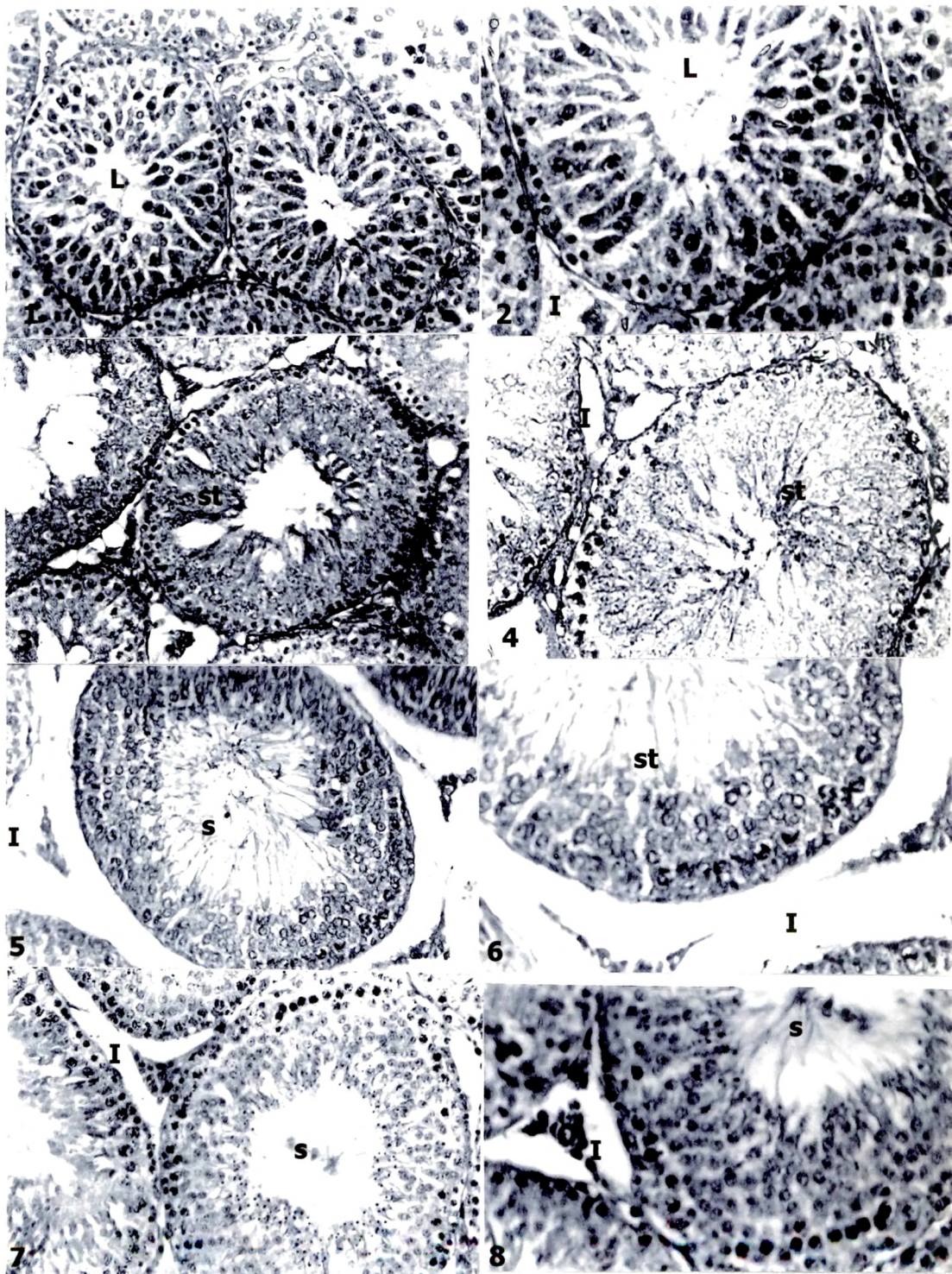
PLATE – I

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:

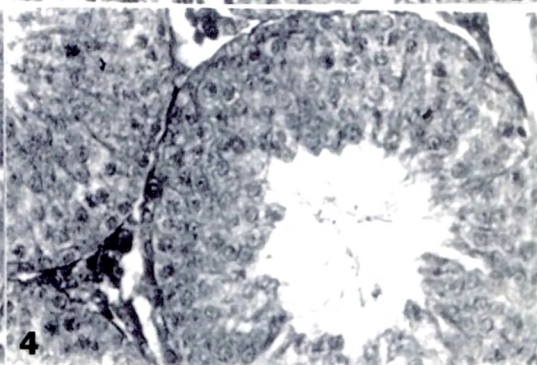I-Interstitium, L-Lumen, st-spermatids, S-sperms,
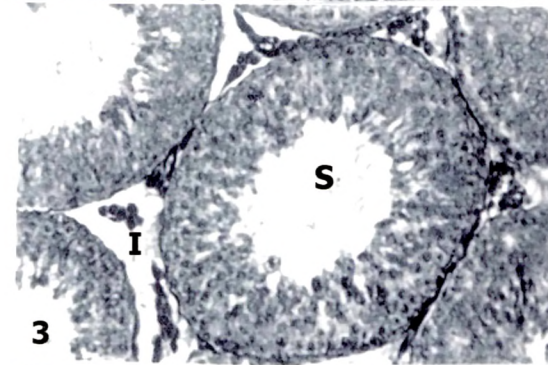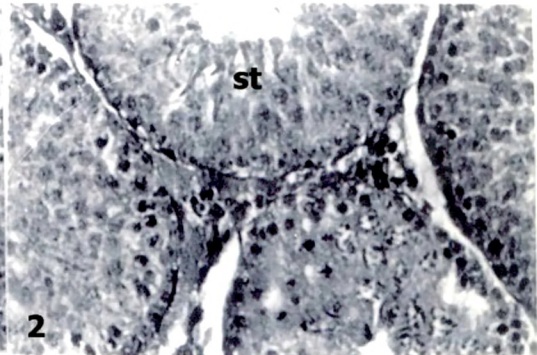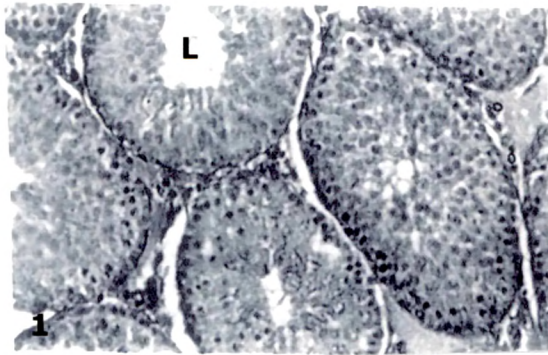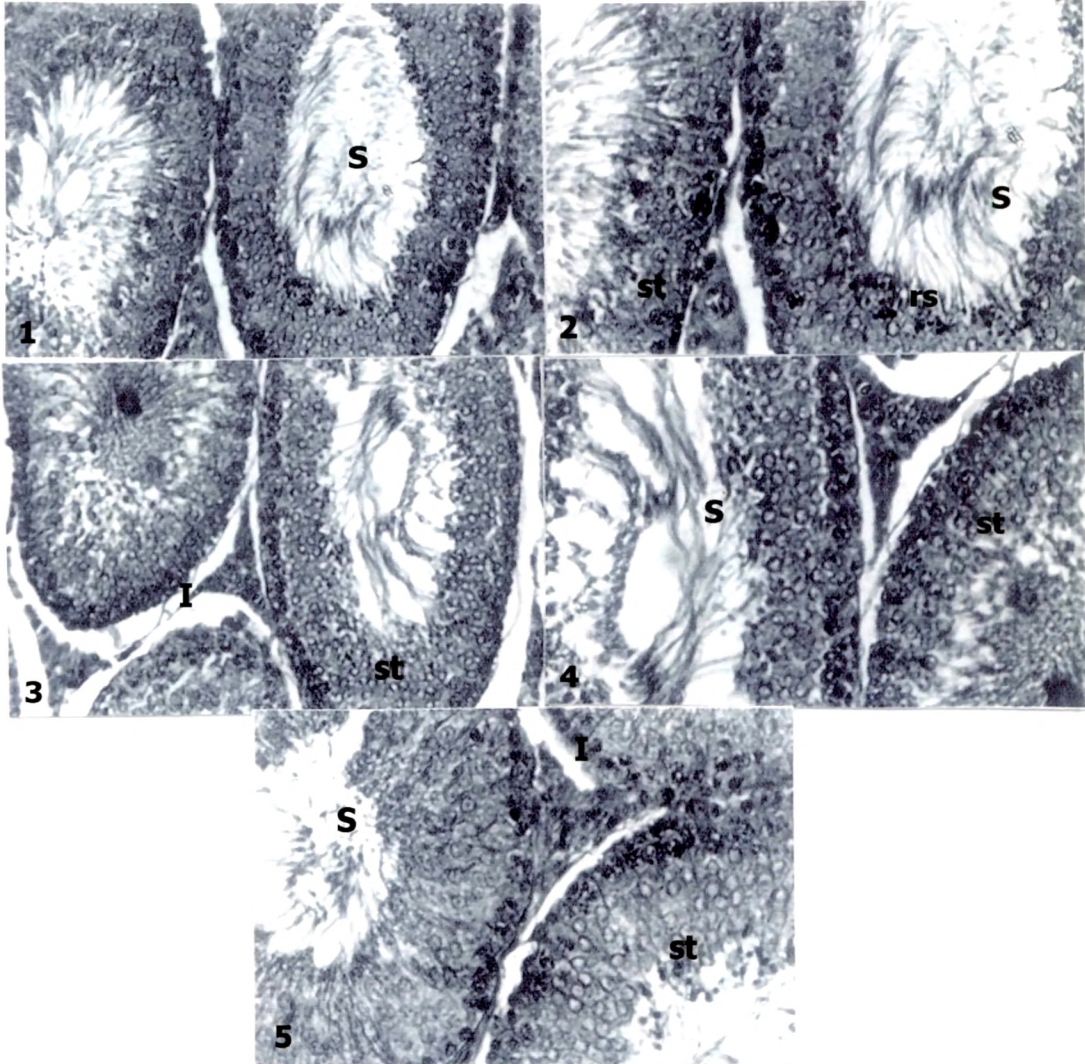D-Degeneration,rs-round spermatids.

PLATE – V a

Figures 1 – 6: Photomicrographs of sections of testis treated with corticosterone in rats.

Figures 1 and 2   :   Sections of testis of 35 day old CE rats showing formation of lumen in some tubules.

Figures 3 and 4   :   Section of testis of 45 day old CE rats showing more number of germ cells and sperms.

Figures 5 and 6   :   Section of testis of 60 day old CE rats showing fully established spermatogenesis and less number of sperms.

CE – High evening Corticosterone injection

Figures: 1, 3 and 5– 250 x
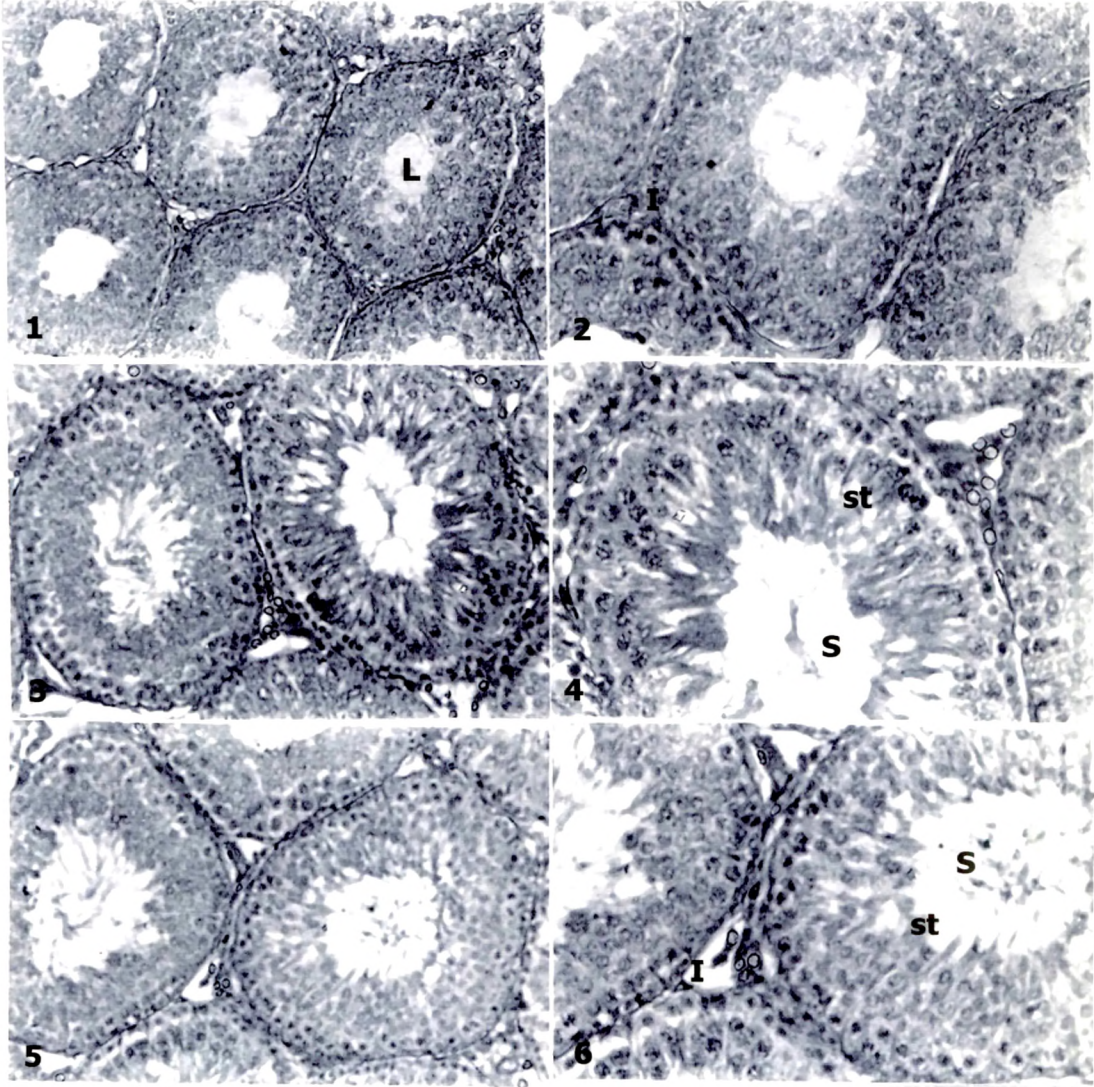**Figures: 2, 4 and 6 – 400 x**
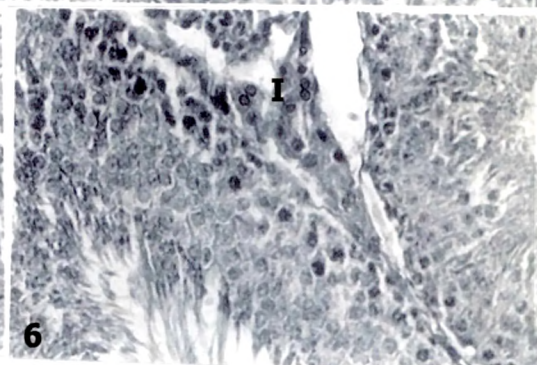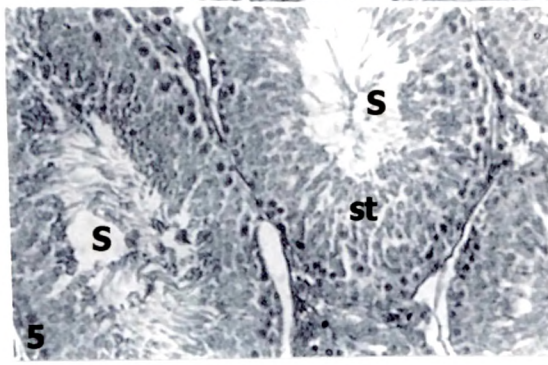
PLATE –V b

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

Figures 1 to 5   : Section of testis of 90 day old CE rats, showing less
populated sperms and early sloughing off of advanced
stages of germ cells.

CE – High evening Corticosterone injection

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

PLATE – VI a

Figures 1 – 6: Photomicrographs of sections of testis treated with
corticosterone in rats.

Figures 1 and 2 : Sections of testis of 35 day old CM rats showing, advanced
stages of spermatogenesis, more number of germ cell and
less degeneration.

Figures 3 to 6 : Section of testis of 45 day old CM rats showing fully
established spermatogenesis, prominent interstitium and
sperms in the lumen

CM – High morning Corticosterone injection

Figures: 1, 3 and 5– 250 x
Figures: 2, 4 and 6 – 400 x

PLATE –VI b

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

Figures 1 to 4   : Section of testis of 60 day old CM rats, showing well formed interstitium and fully established spermatogenesis.

CM – High morning Corticosterone injection

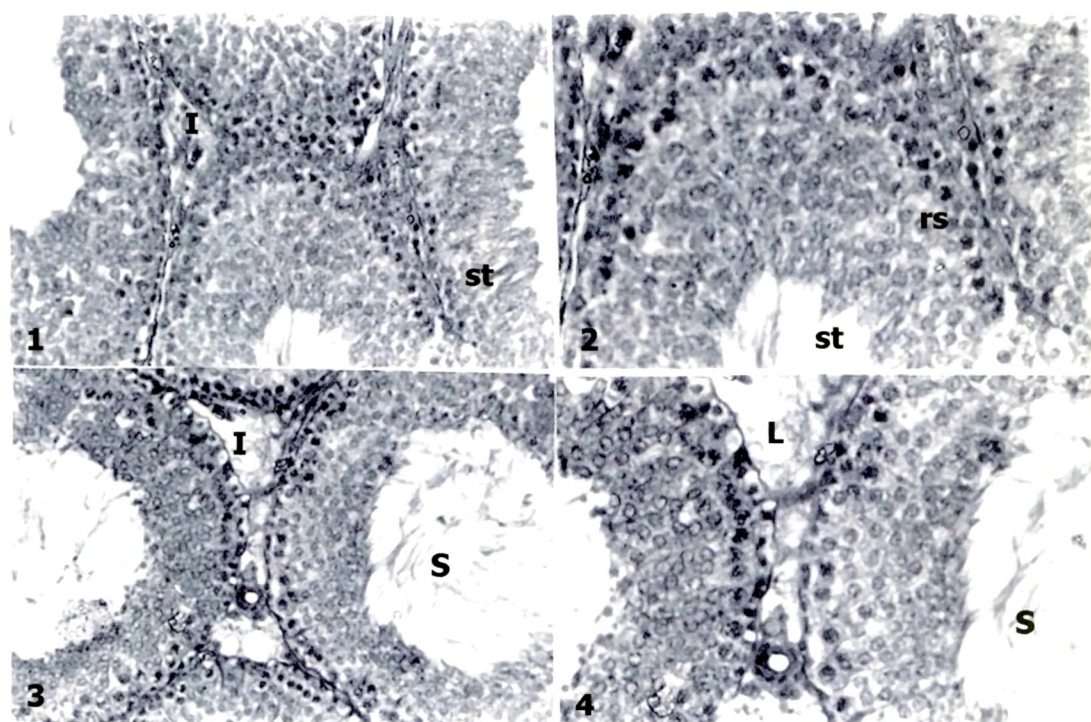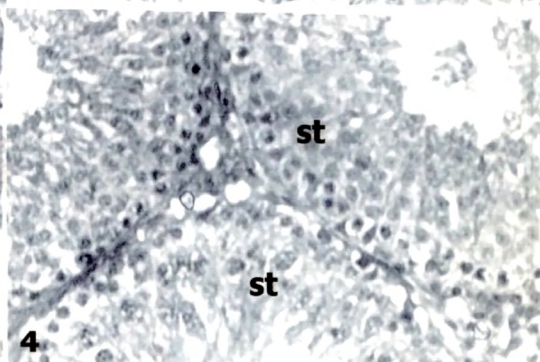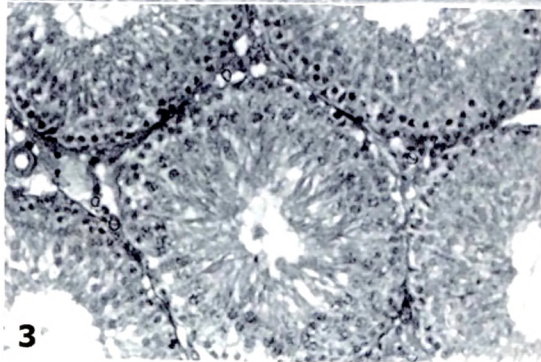**Figures: 1 and 3 – 250 x**
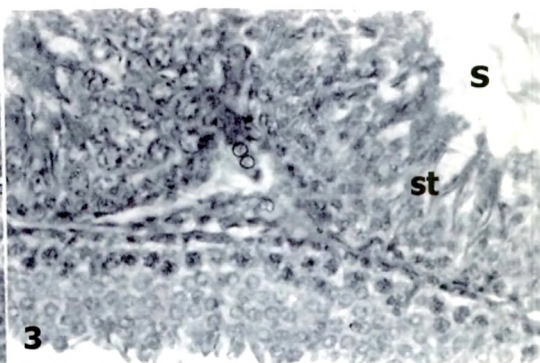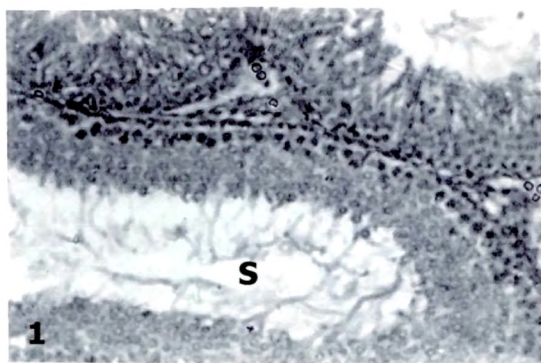**Figures: 2 and 4 – 400 x**

PLATE –VI c

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

Figures 1 to 4 : Section of testis of 90 day old CM rats, showing more number of germ cells and degeneration.

CM – High morning Corticosterone injection

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

SUMMARY

A previous study had assessed the impact of low dose of corticosterone during the neonatal period on adult testis structure and function and hormonal profiles. In the present study a doubled dose of Corticosterone (2 µg per day/ animal from day 0 to day 10 and 4 µg per day/animal from day 11 to day 21) either in the morning (CM) at 0800 hrs in the evening (CE) at 1600 hrs has been tried out. The body weight of CM rats was lower at 90 days and absolute testis weight of CE rats was higher. However there is no difference in relative weight of testes. Puberty onset and establishment of spermatogenesis were both hastened in the experimental groups. The testis and tubular volume and total basement area were all increased in CE animals and decreased in CM animals. There was no significant difference in Sertoli number and tubular length. Though there was increased number of germ cells in experimental rats, the germ cell number per testis and number per meter length of the tubule are marginally higher only in CE rats. This difference is due to higher percentage of germ cell lost in CM rats though, sloughing off of spermatids and spermatozoa is a common feature. The serum corticosterone, LH and T levels were significantly lower in both the experimental group of animals at 90 days. Whereas serum TSH, T_4 and T_3 levels were subnormal in CE animals, the same were above normal in CM animal. These observation have been taken to indicate that exposure to

higher glucocorticoid level in neonatal period can hastened puberty, augment

spermatogenesis and increase germ cells number by decreasing apoptosis on

a long-term basis though with time dependent subtle deleterious effect on

Sertoli-germ cell association. Neonatal glucocorticoid excess also has

permanent effects on the regulatory set points of adrenal, thyroid and

gonadal hormones.