CHAPTER - II

Postnatal glucocorticoid exposure in the preweanling period hastens spermatogenesis and increases germ cell number: I. Dose dependent effect

Stress induced increase in glucocorticoids is shown to affect testicular function and steroidogenesis (Collu *et al.,* 1994; Orr *et al.,* 1994; Maric *et al.,* 1996; Chatterton *et al.,* 1997). Developmental stages are likely to be more vulnerable to hormonal disturbances, especially glucocorticoids. Both elevation or reduction in glucocorticoids can have profound effects. In this respect, hormonal alterations in the foetal environment have been reported to influence the adult phenotype (Barker, 1994). Glucocorticoids are known to be crucial for the maturation of foetal organ systems (Baxter and Rousseau, 1979). However, exposure of foetuses to excess glucocorticoid has been shown to retard growth and precipitate disease in the adult (Benediktsson *et al.,* 1993; Levitt *et al.,* 1996, Lindsay *et al.,* 1996). These observations have led to the premise that glucocorticoids are involved in the programming of postnatal development of various systems. Foetal exposure to glucocorticoid may also be detrimental for postnatal reproductive development as shown by the delayed onset of puberty in the female

offsprings of mothers subjected to stress (Politch and Herrenkohl, 1984a) or treated with adreno-corticotrophic hormone (ACTH) during gestation (Harvey and Chevins, 1987). On these lines, a recent study involving glucocorticoid excess or insufficiency induced by appropriate treatments in pregnant rats from day 13 to term (day 23) has recorded reduced offspring birth weight and delay in the onset of puberty in females with hormone excess and increased birth weight and advanced puberty onset in male offsprings with hormone insufficiency (Smith and Waddell, 2000). It has been concluded from the above study that foetal exposure to glucocorticoid is an important determinant of the timing of puberty onset in the postnatal life, an effect that is manifested within the normal physiological range of glucocorticoid concentrations.

Such experimental findings raised a logical question of the possible influence of glucocorticoid excess during the postnatal period of development on the adult reproductive system. The present study is an attempt to answer this question and test the hypothesis that glucocorticoids in the preweanling postnatal period may have an influence on the functional maturation of testes and puberty onset. This premise has been tested in the present study by exposing the postnatal rat pups to time dependent glucocorticoid excess starting from day 0 to day 21 (weanling) and assessing puberty onset as marked by testicular spermatogenesis and steroidogenesis. Alterations in the circulating profile of various hormones like corticosterone (CORT), thyroid

stimulating hormone (TSH), tri-iodothyronine (T_3), thyroxine (T_4), leutinising hormone (LH) and testosterone (T) have also been evaluated.

MATERIAL AND METHODS:

Animals and Maintenance:

As in chapter one.

Preparation of Corticosterone:

Corticosterone acetate procured from Sigma Co. USA was weighed and the requisite amount was dissolved in a drop of alcohol and then diluted with 0.9 % saline.

Experimental Protocol:

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

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

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 the morning (0800 hrs) from day 0 to day 21 **(C_M)**.

(iii) Injected *i.p.* with vehicle (0.9% saline) in the evening (1600 hrs) from day 0 to day 21 **(C_E)**.

Group II (Corticosterone treated) **(CORT)**:

Newborn rat pups were injected *i.p.* with Corticosterone (Sigma Chemical Co. USA) in the following doses:

(i) 30 newborn pups were injected with corticosterone 1μg/animal/day in the morning (0800 hrs) from day 0 to day 10 and 2μg/animal/day from 11 to 21 days **(CM)**

(ii) 30 newborn pups were injected with corticosterone 1μg/animal/day in the evening (1600 hrs) from day 0 to day 10 and 2μg/animal/day from 11 to 21 days **(CE)**

Parameters and Methods of Evaluation:

As in chapter one.

Histology and Histometry:

As in chapter one.

Hormone Assays:

As in chapter one.

Puberty Onset:

It was assessed by the capacity for preputial separation, determined by manual retraction of prepuce (see Korenbrat *et al.,* 1977).

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:

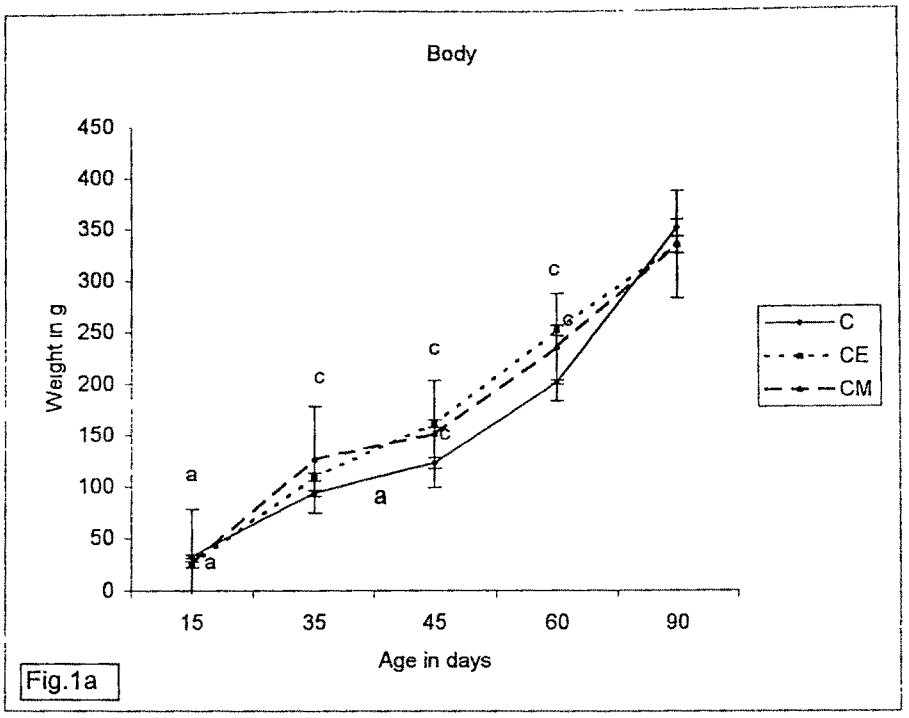
The body weight of the experimental groups (CM and CE) was significantly less during the treatment period (Table 1; Figure 1a). There was a significant compensatory increase during 15-35 days in CM and CE rats. However, the body weight of both CM and CE rats at 90 days was less than the controls. The relative weight of testes which tended to remain lower till 60 days in the experiment groups, became higher at 90 days though, statistically insignificant (Table 1; Figure 2). The overall growth rate was not significantly different, however the growth rate between 0-45 days was higher in the experimental animals with maximal growth between 35 and 45 days as recorded in CE animals (Table 2; Figures 3a & 3b).

Table 1: Chronological alterations in body weight (g) and absolute (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	32.640 ±1 754	93.600 ±2.958	122.5 ±5.155	200.6 ±2.172	349.100 ±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	24 873[a] ±2 720	109.167[a] ±4.053	160.833[c] ±3.439	250.50 [c] ±4.945	332.667 ±10.582	0.218[b] ±0.024	0.984 ±0.047	1.805[c] ±0.063	2.309 ±0.085	3.243 ±0.067	0.895 ±0.048	0.904 ±0.044	1.128 ±0.057	0.921[c] ±0.089	0.967 ±0.020
CM	26 122[a] ±2.549	126.333[c] ±3.575	150 833[c] ±3.380	234.667[c] ±5.536	333 767 ±6.498	0.254[a] ±0.030	1 232[b] ±0.026	1.638[a] ±0.069	2.650 ±0.042	3.223 ±0.024	0.967 ±0.050	0.980 ±0.041	1.090 ±0.057	1.133 ±0.037	0.966 ±0.015

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

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



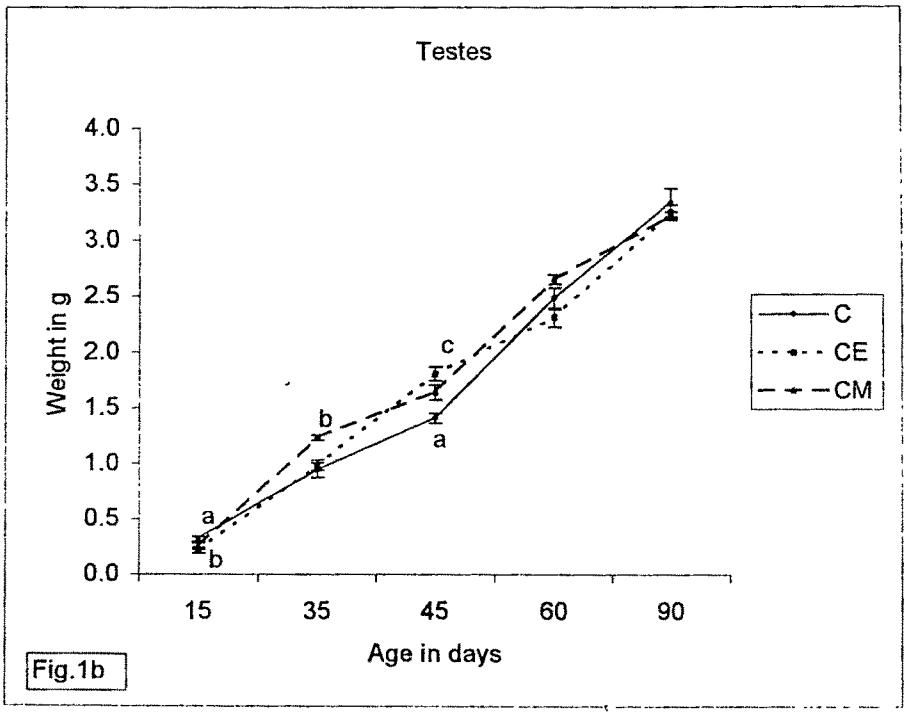
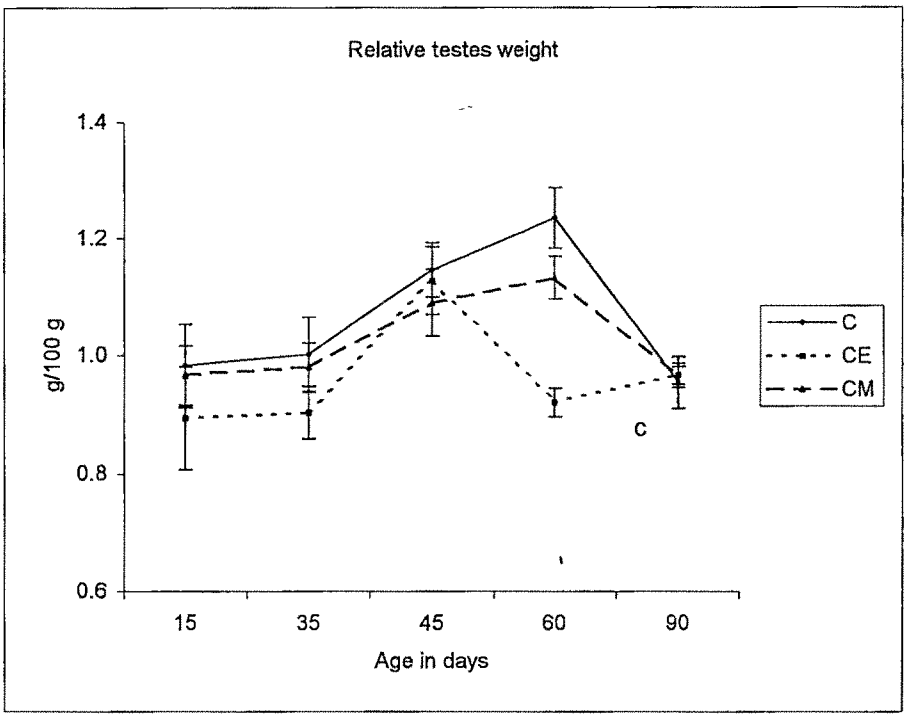


Fig. 1a and 1b. Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Corticosterone treated rats
C - Control, **CE** - Low Dose evening Corticosterone injection, **CM** - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of six animals,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



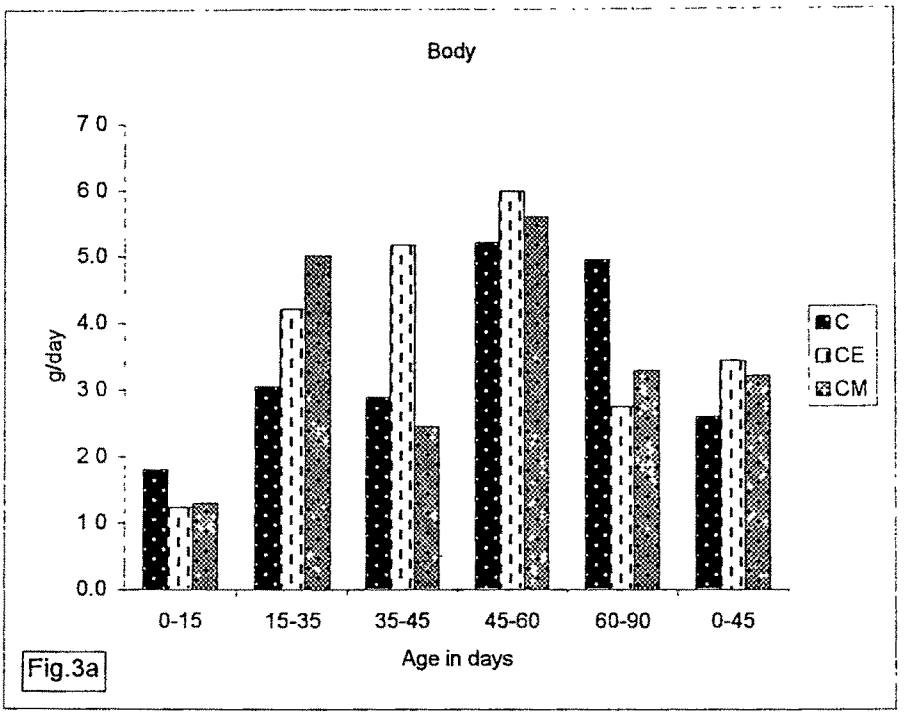
Chronological alterations in relative weight (g/100 g) of testes in Control and Corticosterone treated rats

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] $p < 0.0005$

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	0-45	0-15	15-35	35-45	45-60	60-90	0-45
C	1.804	3 048	2.890	5.207	4.950	2.598	0.021	0.031	0 047	0.072	0.028	0.031
CE	1.234	4.215	5.167	5.978	2.739	3.433	0.014	0.038	0 082	0.034	0.031	0.040
CM	1.300	5.011	2.450	5.589	3.283	3.205	0.017	0.049	0.041	0.067	0.019	0.036

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



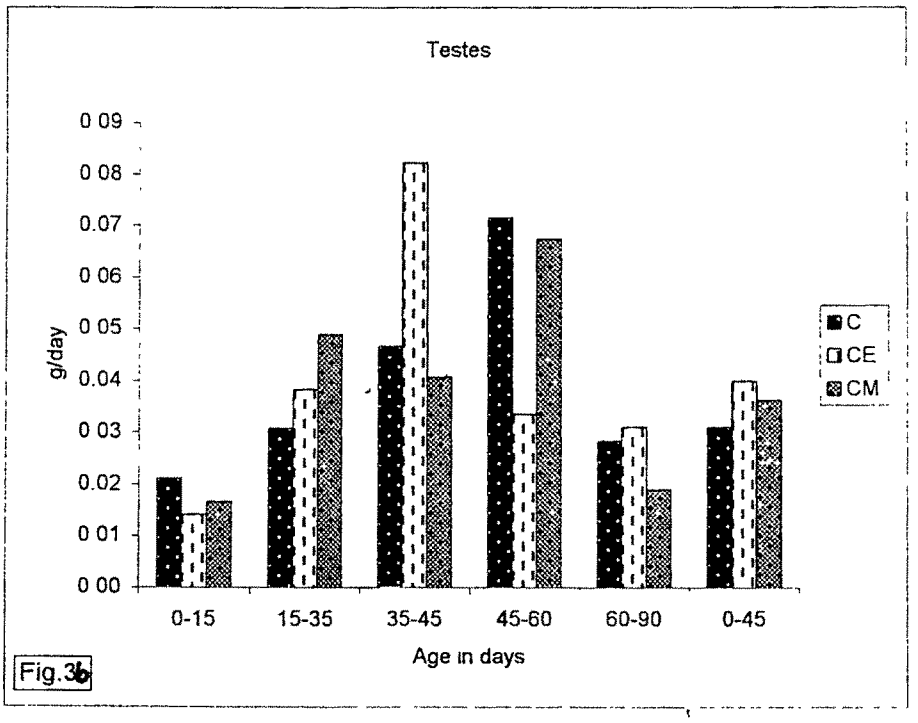


Fig. 3a and 3b Per day Body and Testes Growth rate (g/day) in Control and Corticosterone treated rats

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

Histology and Histometry:

Control testis sections reveal establishment of full spermatogenesis marked by the appearance of sperms by 60 days (Plate 1). However, in CM and CE, establishment of spermatogenesis was hastened as marked by the appearance of sperms by 45 days. At 60 and 90 days, the testis sections of CM and CE animals showed compactly packed increased number of germ cells and, higher sperm mass at 90 days in CE. The interstitial cells were also found to be prominent in the experimental animals. There was significant increase in tubular diameter and germinal epithelial thickness but no increase in Sertoli cell count in the experimental groups (Table 3). In fact the number of Sertoli cells showed a significant decrement, more with the morning regimen. The tubular length was significantly decreased in CM rats and the total basement membrane area was increased in CE rats. The total germ cell number per testis, both theoretical and actual were both significantly higher in CM and CE animals but, relatively more in CE. But the germ cell number per meter length of the tubule was more in CM than in CE rats. The total germ cell loss was significantly more in CM than in CE, but much lesser than in the controls (2% in CE, 7% in CM and 10% in C) (Table 3) (Plates IIIa, IIIb, IVa, IVb and IVc.)

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 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	**1.775** ±0.160	0.0310^b ±0.0007	0.0100^c ±0.0002	1.668 ±0.150	2214.16 ±95.200	215.441^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
CM	**1.471** ±0 050	0.0340^c ±0.0005	$0 0110^c$ ±0.0003	1 398 ±0.040	1553.68^c ±65.100	$165 208^c$ ±3.650	$22 02^c$ ±1.600	342.0^b ±8.600	318.0^b ±8 900	22.02^c ±0.360	$20 47^c$ ±0.980	7.030 ±1.790

C – Control, **CE** – Low Dose Corticosterone evening injection, **CM** – Low 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.

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	21 750 ±0 854	48 125 ±1.235	53.250 ±1 031	0.235 ±0 019	0.550 ±0.166	2.325 ±0.278	2.625 ±0.217	4.375 ±0.265
CE	13 10^{c} ±0 653	15.600^{c} ±0 208	13 245^{c} ±0 132	10.045^{c} ±0.522	9.100^{c} ±0 227	11.768^{c} ±0.094	20.575^{b} ±0.776	10.650^{c} ±1.028	14.000^{c} ±1.135	26.875^{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
CM	23 97^{c} ±0 168	34.1^{c} ±0.246	48.150^{c} ±0.171	10 500^{c} ±0.041	9.968^{c} ±0 198	13.705^{c} ±0.137	18.600^{a} ±0.524	13.750^{c} ±1.031	18.025^{c} ±1.065	38 358^{c} ±0.620	0 350^{b} ±0.019	1.720^{b} ±0.211	1.250^{a} ±0.263	1.750^{b} ±0.087	2.108^{b} ±0.134

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

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

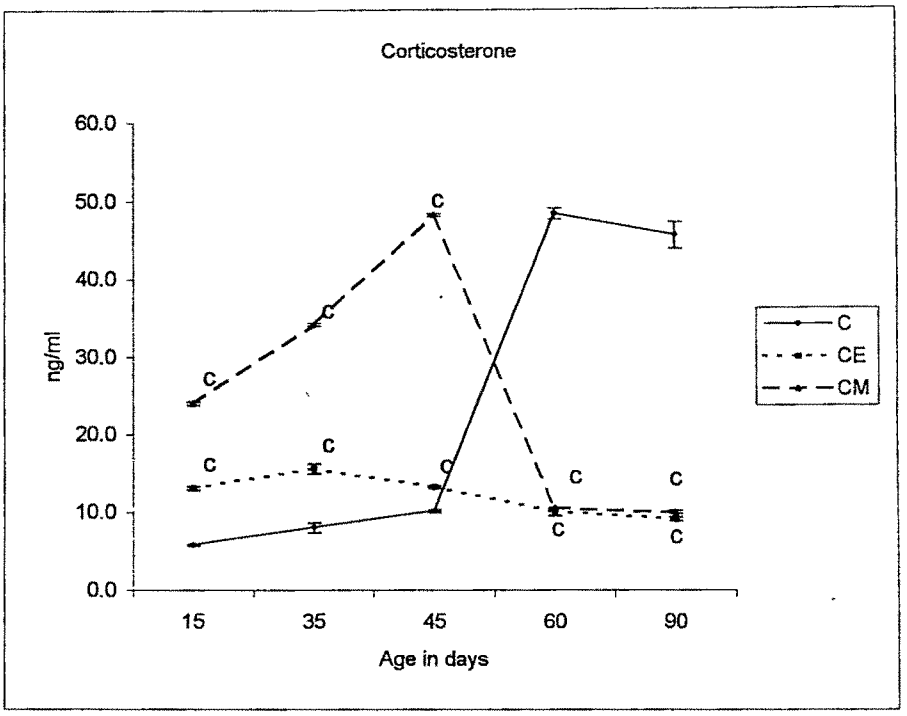
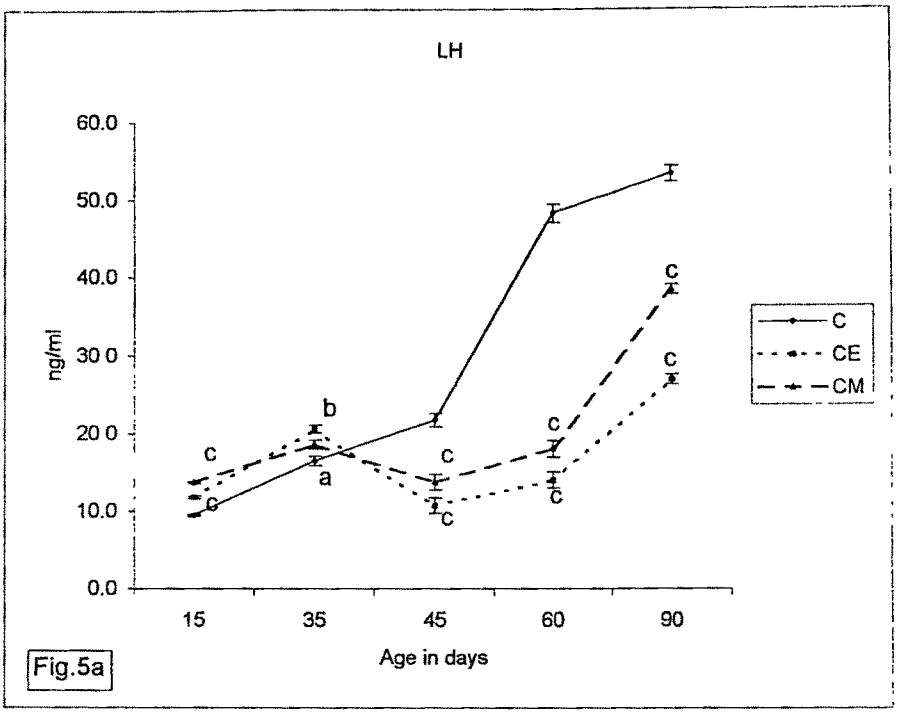


Fig.4: Serum Corticosterone level (ng/ml) in Control and Corticosterone treated rats

C - Control, CE - 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] $p < 0.0005$



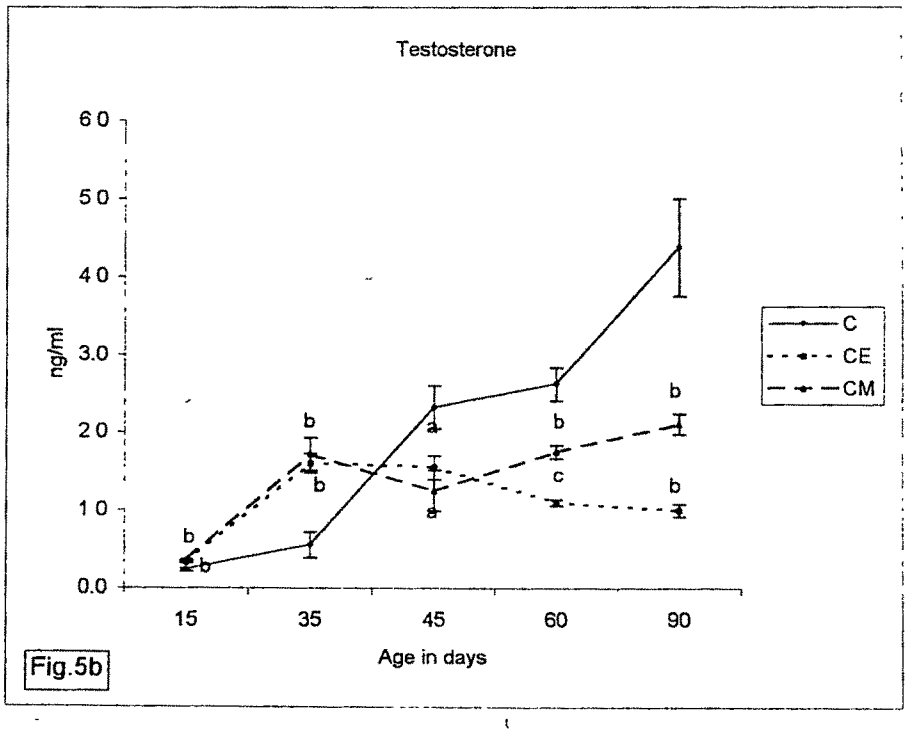


Fig.5a and 5b: Serum LH and T levels (ng/ml) in Control and Corticosterone treated rats

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] $p < 0.0005$

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	3.470 ±0.137	6.170[a] ±0.144	7.283[a] ±0.176	7.633 ±0.062	7.823[c] ±0.125	0.465[b] ±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[b] ±0 031	0.734[c] ±0.086	1.053[b] ±0.056	0.400[b] ±0.082
CM	3 225 ±0 155	6.265[a] ±0 105	8.048[c] ±0.065	7.570 ±0.125	8.675[c] ±0.085	0 400 ±0.108	0.793[a] ±0 067	1.550[a] ±0.131	1.775[c] ±0.069	2 823 ±0.092	0.285 ±0.060	0.523 ±0.104	0.403 ±0.034	0.708 ±0.086	0.840[b] ±0.039

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

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

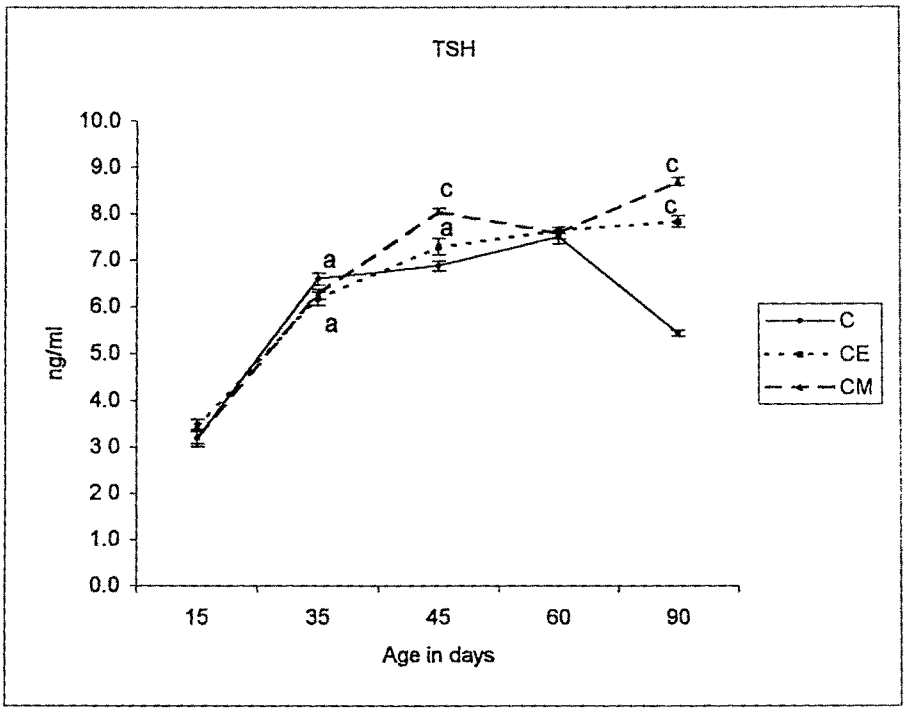
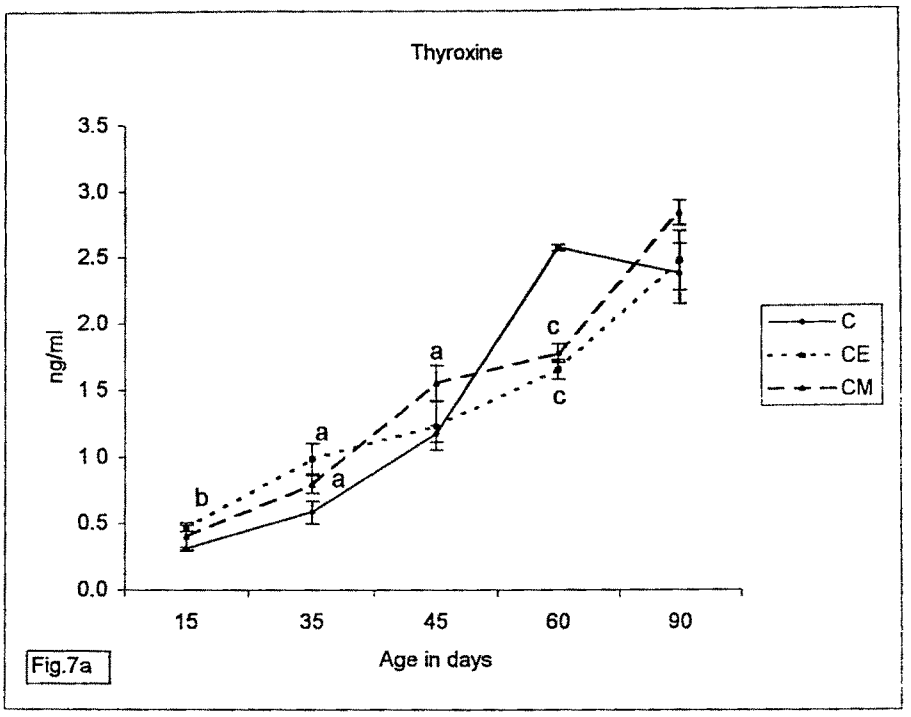


Fig.6: Serum TSH level (ng/ml) in Control and Corticosterone treated rats
C - Control, **CE** - Low Dose evening Corticosterone injection, **CM** - Low Dose morning Corticosterone injection, Values expressed as Mean ± SEM of four samples, ^{a} $p< 0.05$, ^{b} $p< 0.005$, ^{c} $p< 0.0005$



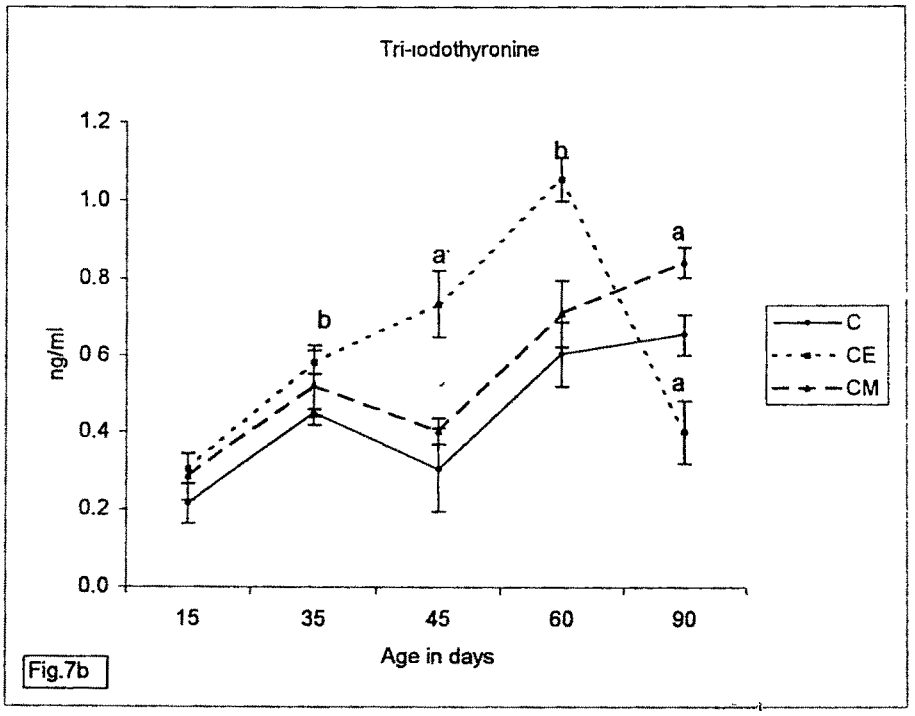


Fig.7a and 7b: Serum T_4 and T_3 levels (ng/ml) in Control and Corticosterone treated rats

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 Hormone Profile:

Corticosterone:

Serum corticosterone levels were higher in CM and CE animals throughout the treatment period as well as subsequently till 45 days. Thereafter there was permanent significantly reduced corticosterone titre in the adult condition (60 and 90 days) (Table 4; Figure 4).

TSH, T_4 and T_3:

TSH, T_4 and T_3 levels were by and large significantly higher in the experimental animals throughout including treatment period and in the post-treatment period extending to the adult stage (Table 4; Figures 6, 7a & 7b).

LH and Testosterone:

LH and T levels were significantly higher during the treatment period and this trend persisted till 35 days. Thereafter the levels of LH and T remained consistently and significantly lower in the experimentals as compared to controls (Table 4; Figures 5a & 5b).

DISCUSSION:

The present investigation indicates that postnatal response to glucocorticoids can affect the subsequent timing of puberty and quality and quantity of spermatogenesis in the adult condition. Though deleterious effect of excess corticosterone in the adult stage has been documented

(Benedicktsson *et al.,* 1993; Levitt *et al.,* 1996; Lindsay *et al.,* 1996), no report is available with regard to neonatal corticosterone excess on puberty onset or on testis functions. This is the first report, which shows a favourable influence of excess corticosterone in the physiological range during neonatal preweanling period on puberty onset and spermatogenesis. This study also indicates that though both morning and evening exposure to corticosterone is effective, evening exposure coinciding with the endogenous rise as per the known diurnal corticosterone rhythm (Poland *et al.,* 1981) is relatively more favourable. This is attested to by the increased body and testes weights in the immediate post exposure periods. Though there is no significant difference in the body and testes weights at 90 days, there was hastened growth dynamics as seen by the higher body and testes weights at 35 and 60 days. The relative weight of testes at 90 days tended to be higher though statistically insignificant (Table 1; Figure 2). These aspects are in contrast to the observed birth weight and attainment of puberty in rats exposed to either an excess of or deficient glucocorticoid in the foetal period, with the former resulting in decreased birth weight and delayed onset of puberty and the latter in increased birth weight and earlier onset of puberty (Benedicktsson *et al.,* 1993; Burton and Waddell, 1994; Lindsay *et al.,* 1996; Smith and Waddell, 2000). Obviously, there is differential sensitivity to glucocorticoid in relation to foetal or postnatal development.

It is clear from the data (Table 2) that there is retardation in body growth rate during the treatment period but the post-treatment periods upto 60 days, generally characterized by increasing growth rate, was marked by significantly pronounced rates in the CE & CM groups of animals. This temporally hastened growth manifestation was also clearly reflected in the testes growth between days 0 to 45 (0.03 C v/s 0.040 CE and 0.036 CM). Much of this growth was between 35 and 45 days in CE and between 45 and 60 days in CM. The positive influence of transient neonatal exposure to corticosterone was also manifested in the form of early attainment of puberty (42 days in CM and CE v/s 50 days in controls).

The temporally enhanced growth dynamics is further confirmed by the advanced onset of spermatogenesis and appearance of sperms by 45 days in the testis and epididymis. The same occurred in the control rats later than 45 days. Besides hastened spermatogenesis, the testes of CE and CM rats were also marked by significantly higher density of germ cells at all ages. The tubules of CE testis also showed a dense population of spermatozoa at 90 days, further underscoring the relatively more favourable influence of evening exposure to excess corticosterone than morning exposure (Plate 1). Despite reduced tubular length and reduced Sertoli cell number, the significant greater number of germ cells (both theoretical and actual) indicate reduced germ cell loss by apoptosis, more so with the evening schedule (Table 3). However, morning corticosterone seems to show greater germ cell

loss by degeneration compared to evening treatment. The degenerative loss in CE animals seems relatively closer to that of control rats. Apparently, neonatal evening corticosterone excess is more favourable for overall germ cell survival, which contributes to an increased germ cell population. Due to the significant decrease in tubular length, the number of germ cells per meter length of tubule was higher in CM rats. Corticosterone treatment in general, irrespective of the time of administration, is favourable compared to controls. Another interesting observation is that major part of the growth involving tubular length is completed by 35 days (65% approximately) in both CE and CM animals, thereby alluding to a role for corticosterone in promoting tubular elongation. A very interesting observation is the presence of only two or three stages of spermatogenesis in all tubules of a section, more often stages V-VII. It is a matter of speculation as to whether corticosterone treatment in the neonatal period, more specifically evening schedule, synchronises initiation of spermatogenesis for longer stretches in the tubule which should, as a consequences, reduce the number of waves per tubule. This aspect needs critical evaluation for necessary validation. The observed difference in germ cell population and sperm density in the corticosterone-exposed animals is amply validated by the recorded higher relative weights of testes at 90 days.

Possible mechanisms that may account for the observed hastened growth dynamics, early onset of puberty and temporally advanced

spermatogenesis with increased germ cell density, may become meaningful when viewed against the recorded alterations in circulating titres of hormones. Neonatal corticosterone exposure seems to have significant effects on many hormonal axes directly. Corticosterone excess during the neonatal period appears to have a long term depressing influence on the set point of the hypothalamo-hypophyseal-adrenal (HHA) axis, as marked by the lower levels of corticosterone titre in the adult (60 to 90). An earlier study in neonatal deprivation for a day (day 11) showed higher corticosterone response to stress as adults without altering the basal level (Suchecki and Tufik, 1997). Preceding this basal level, the corticosterone levels in the pubertal and pre-pubertal periods (35 and 45 days) were higher. Apparently, the increased corticosterone level in the treatment period persisted and extended upto atleast a month after the cessation of treatment. It may be speculated that neonatal chronic exposure to corticosterone decreases the metabolic clearance of the hormone leading to elevated level, an effect, which persists for sometime after exposure to corticosterone. An opposite permanently elevating influence on the hypothalamo-hypophyseal-thyroid (HHT) axis can also be inferred by the recorded high circulating levels of T_3, T_4 and TSH. Previous studies on neonatal thyroid hormone status during the preweanling period have clearly established the role of T_3 in inducing Sertoli cell differentiation and conversely, prolonged Sertoli cell proliferation and consequent increase in number due to hypothyroidism (Hess and Cooke,

1992; Van Haaster *et al.,* 1992; Hess *et al.,* 1993; Cooke *et al.,* 1994; Franca *et al.,* 1995). The presently observed higher T_3 and T_4 levels during the critical window of Sertoli cell proliferation in this context portend augmented Sertoli cell differentiation. This stands well correlated by the observed temporally advanced onset of spermatogenesis and consequent appearance of sperms by postnatal day 45 in the CE and CM rats.

The levels of LH and T were significantly higher till 35 days followed by persistently lower levels in the pubertal and adult stages. Apparently neonatal corticosterone excess for a sufficiently longer period seems to have a dampening influence on the hypothalamo-hypophyseal-gonad (HHG) axis. Presumably, neonatal corticosterone exposure lowers the set point of the HHG axis. Prominent 3β and 17β-HSDH activities observed in the CM and CE rats at 35 days support the higher T level favouring hastened spermatogenesis (not included in this study). Such reprogramming of hormonal axes by neonatal glucocortoid exposure is tenable in the context of reported actions of foetal glucocorticoid exposure on the HHG axis (Smith and Waddell, 2000) as well as on the HHA axis (Meaney *et al.,* 1991). Since glucocorticoid receptors have been shown in the neural tissue (Reagan and McEwen, 1997; Daikoku and Koide, 1998), pituitary gonadotrophs (Kilen *et al.,* 1996), the ovary (Michael *et al.,* 1993) and testis (Monder *et al.,* 1994), reprogramming modulations by glucocorticoid in the neonatal period cannot be discounted.

The increased germ cell population and sperm density in adult rats exposed to corticosterone neonatally, are novel features, which require some explanation. One possible reason for the increased germ cell number is an increase in the number of Sertoli cells as manifested in rats rendered hypothyroid neonatally (Cooke *et al.,* 1994). But this is also related with an overall increase in tubular diameter as well as testis size and weight. The latter effect is not manifest in the present case and in fact there is decrement in Sertoli cell number especially in CM rats. Arguably, the increased germ cell population seen in the present study is not due to an increase in Sertoli cell number but due to an actual decrease in the quantum of germ cell loss. This is confirmed by the histological observations of decreased germ cell degeneration at all ages compared to controls. The germ cell loss occurring during spermatogenesis as a normal event is now clearly established as apoptosis and, has been estimated to result in the loss of upto 75% of germ cells (Huckins, 1978; deRooij and Janssen, 1987; Blanco-Rodriguez, 1998). The reduced germ cell loss in the present study results in relatively more number of germ cells being supported by each Sertoli cell. It is speculated from the present circumstantial evidences that neonatal exposure to corticosterone excess somehow attenuates the normal rate of germ cell apoptosis probably, by way of altered pattern of secretion of growth/paracrine factors and/or adhesion molecules from the Sertoli cells by a permanent genetic reprogramming. This is understandable in the context of

reported presence of corticosterone receptors in the testes as well as the non-expression of 11β-HSDH (a metabolising enzyme of corticosterone) in the preweanling period (Hardy *et al.,* 1998) which could result in hyper stimulation and thereby altered Sertoli cell expression. Though glucocorticoids have been known to promote apoptosis in many tissues (Gonzalo *et al.,* 1993; Hassan *et al.,* 1996; Waddell *et al.,* 2000), the presently revealed anti-apoptosis action in the testis is validated by the observation of an inhibitory role of glucocorticoids on apoptosis in neutrophils leading to increased neutrophil survival (Cox, 1995) and also in glomerular endothelial cells (Messmer *et al.,* 1999).

In conclusion, this study reveals that excess glucocorticoid exposure during the preweanling neonatal period hastens puberty, augments spermatogenesis and increases germ cell number and sperm density essentially by decreasing germ cell apoptosis on a long-term basis in the adult.

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.

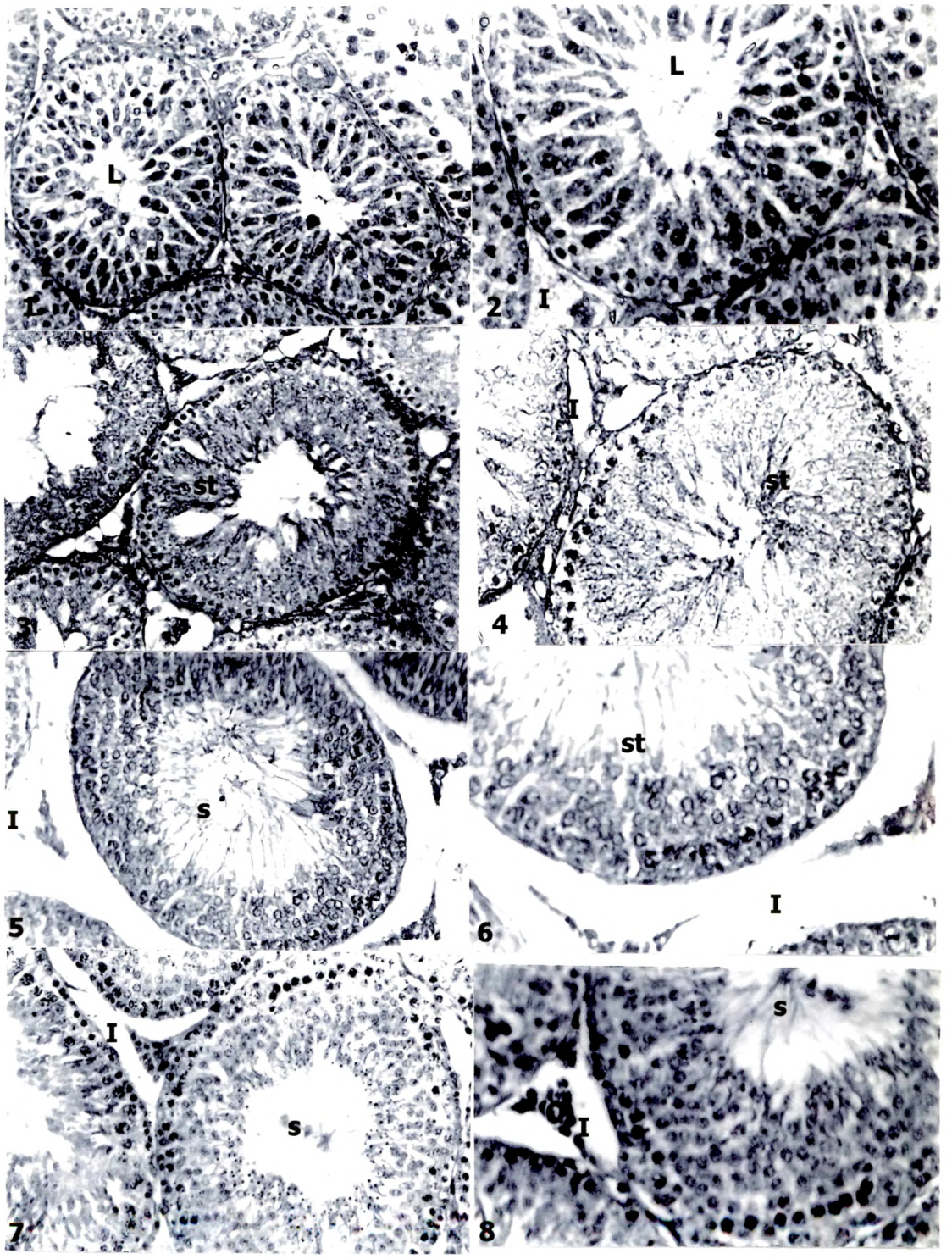

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PLATE – III a

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

Figures 1 and 2 : Sections of testis of 35 day old CE rats showing inhibited completion of meiosis.

Figures 3 and 4 : Testis section of 45 day old CE rats showing, fully established spermatogenesis and thinner population of sperms and more number of germ cells.

CE – Low evening Corticosterone injection

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

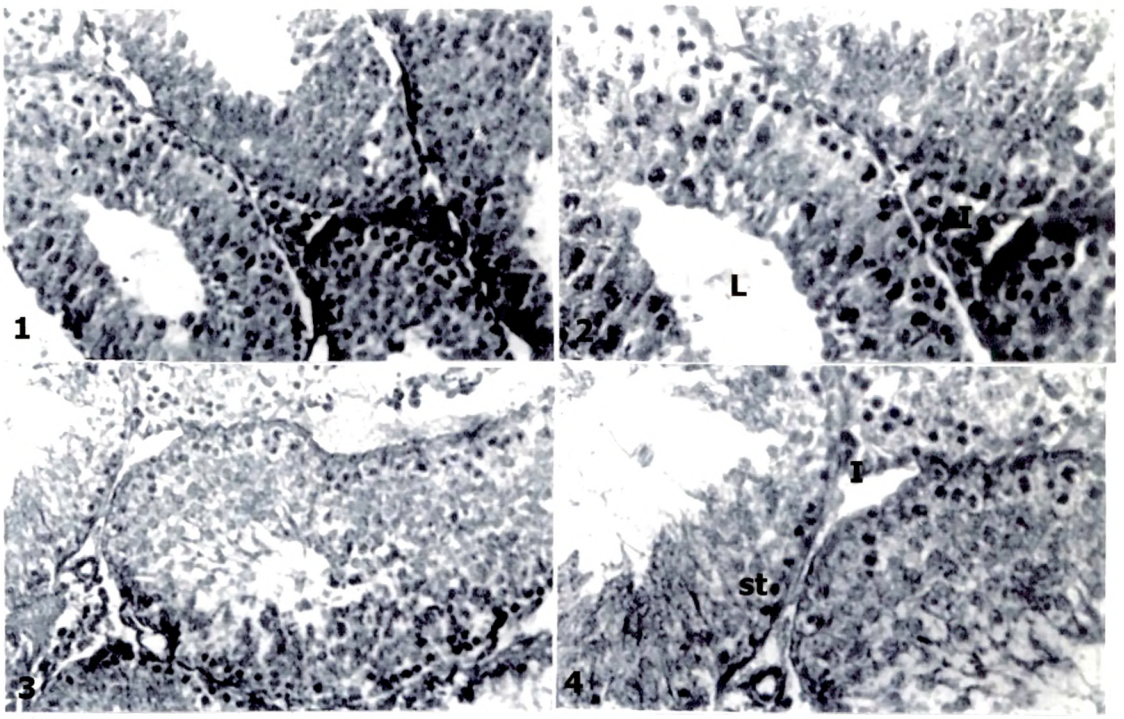
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PLATE – III b

Figures 1 – 7: Photomicrographs of sections of testis of rats treated with corticosterone.

Figures 1 to 4 : Testis section of 60 day old CE rats showing sperms and more number of germ cells.

Figures 5 to 7 : Testis section of 90 day old CE rats showing, high content of sperms and less number of germ cells. Prominent interstitium with large number of cells but smaller in size.

CE – Low evening Corticosterone injection

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

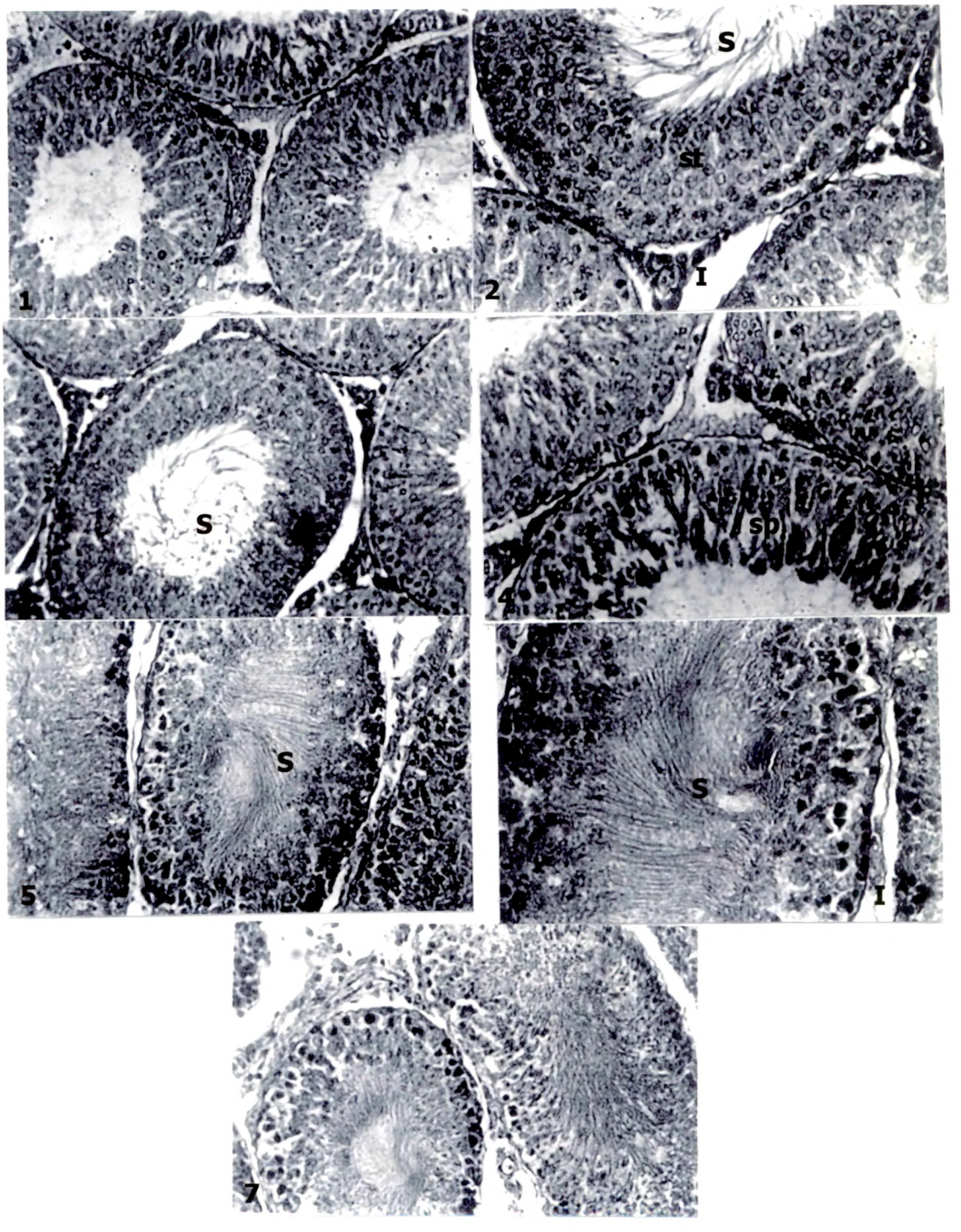
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PLATE – IV a

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

Figures 1 and 2 : Section of testis of 35 day old CM rats, showing sperms hypertrophied interstitium, elongating spermatids and more number of germ cells.

Figures 3 to 6 : Section of testis of 45 day old CM rats showing sperms and more number of germ cells.

CM – Low morning Corticosterone injection

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

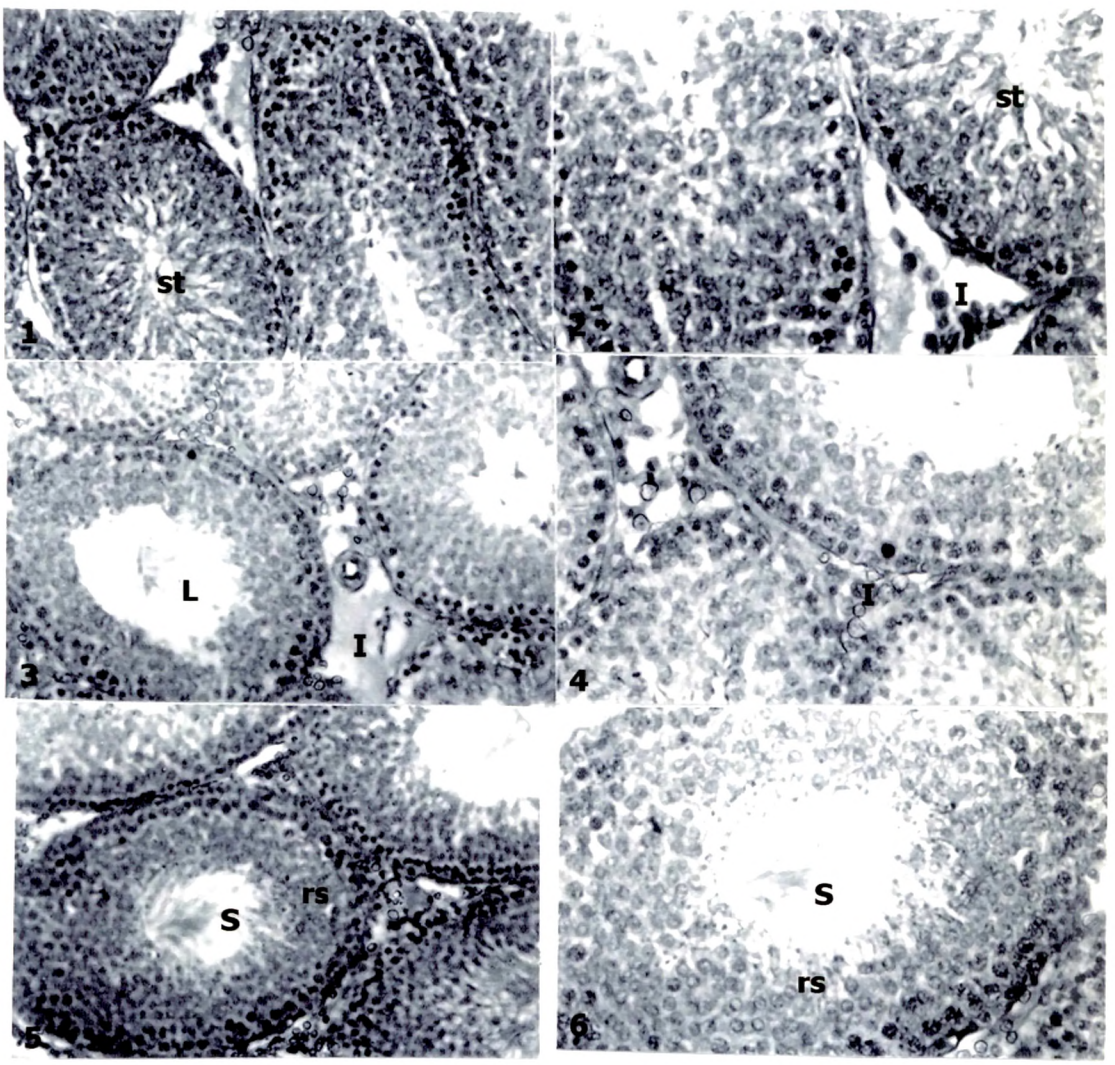
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PLATE – IV b

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

Figures 1 to 5 : Section of testis of 60 day old CM rats, showing larger tubules and large number of germ cells.

CM – Low morning Corticosterone injection

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

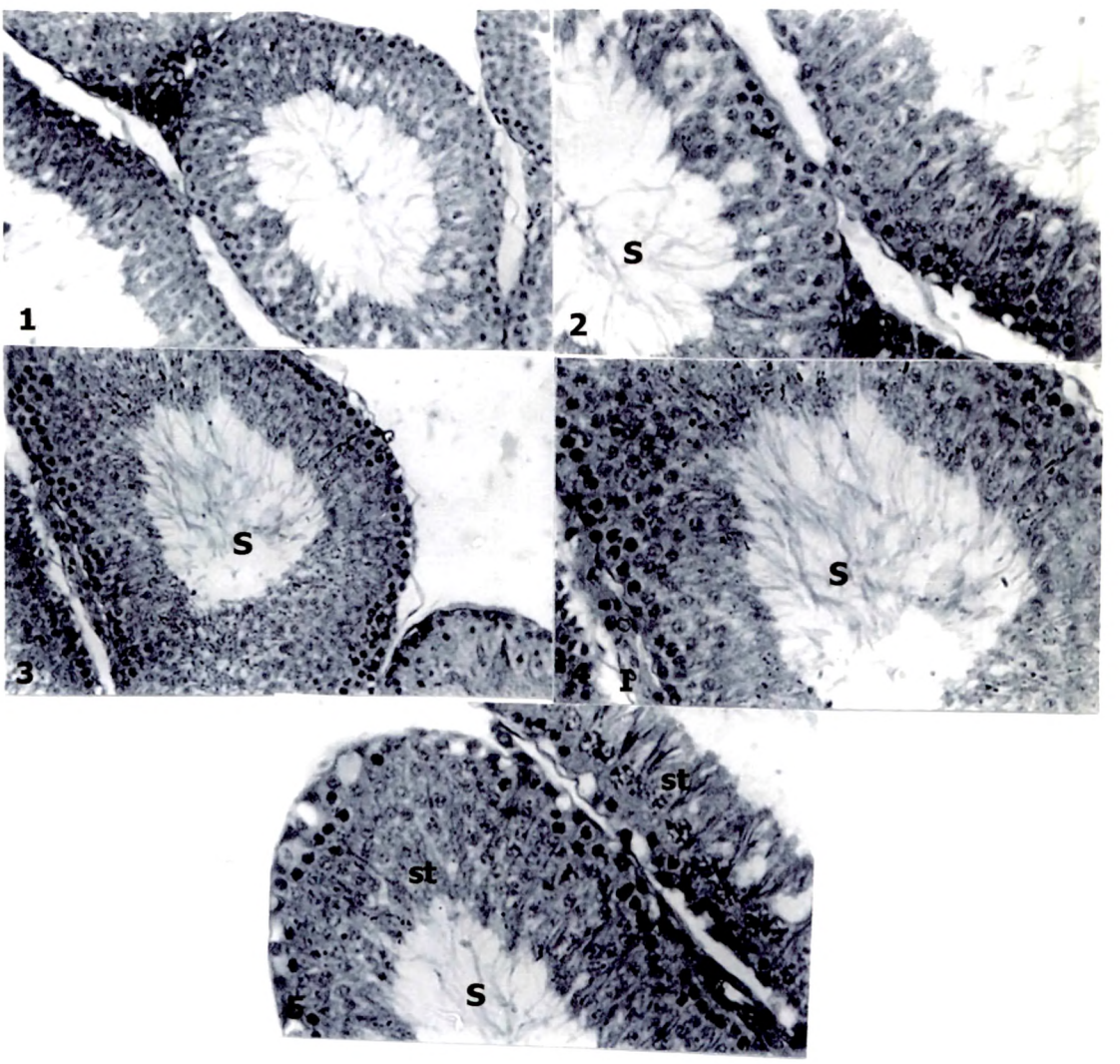
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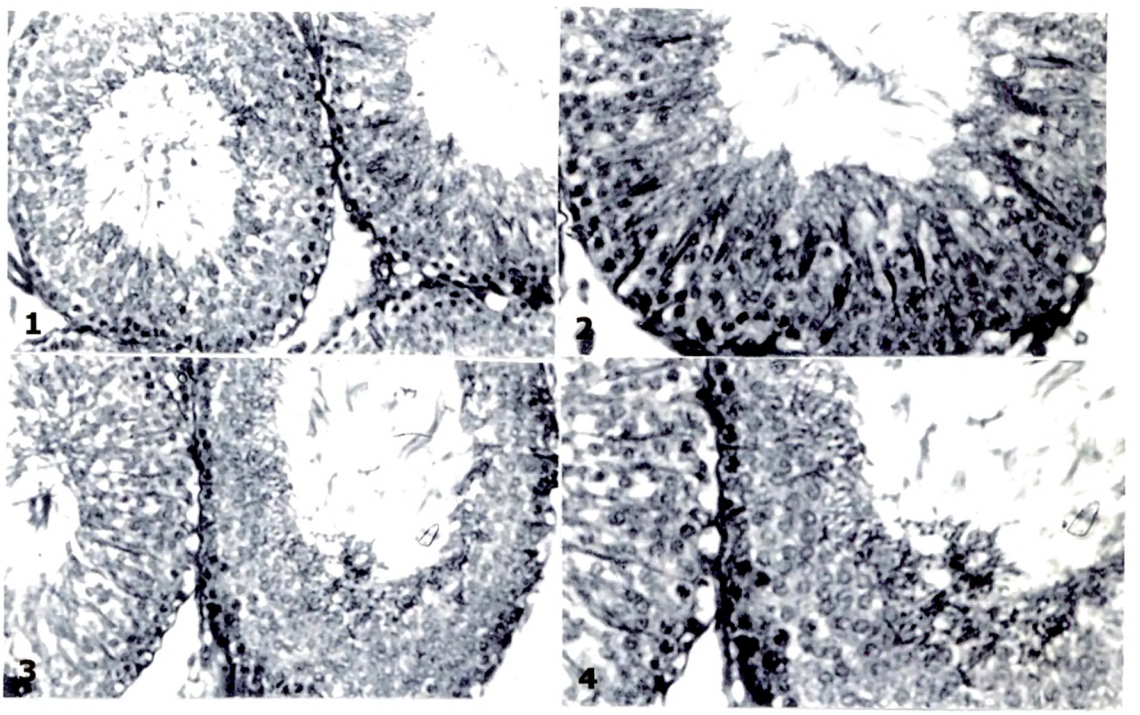
PLATE – IV c

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.

CM – Low morning Corticosterone injection

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

Time dependent effect of mild glucocorticoid excess during the neonatal period has been accessed on adult testis function and on the hormonal status of TSH, T_4, T_3, LH, T and CORT by the administration of corticosterone either in morning at 0800 hrs or in the evening at 1600 hrs (1μg / day/ animal from day 0 to day 10 and 2 μg /day/ animal from day 11 to day 21). The treatment had no significant effect either on body weight or testes weight at 90 days of age, though the body weight was significantly low during treatment period. The experimental animals showed early puberty onset and hastened spermatogenesis marked by the appearance of sperm by 45 days in the testis tubules. The germ cell number was increased with increase in tubular diameter and germinal epithelial thickness with compactly packed cells. There was decrease in total tubular length in CM rats and increase in total basement area in CE rats and decrement in Sertoli cells in both. The germ cells number though higher in both the experimental groups was nevertheless relatively more in CE. The total germ cell loss was much lesser than in controls but relatively more in CM comparison to CE. The adult serum TSH, T_3 and T_4 levels were significantly higher in experimental rats while the corticosterone level was significantly lower. The serum LH and T were levels also significantly lower, though their levels were initially higher along with corticosterone during the treatment and immediate post-treatment

period. The recorded observations suggests that neonatal corticosterone excess is favourable for puberty onset and establishment of spermatogenesis. There is significant effect on germ cell number by probably decreasing germ cell apoptosis. There is retardatory influence on tubular length increase, total basement area and Sertoli cells, more specifically in CM rats. The total number of germ cells per testis is significantly higher in CE rats but the number per meter length of tubule is higher in CM rats and the number of germ cells supported by each Sertoli cell is higher in the experimental rats. It is hypothesized that neonatal hypercorticalism decreases germ cell apoptosis by genetic reprogramming of the Sertoli cell secretory function.