Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2010, 2 (5):269-284

(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Neonatal glucocorticoid exposure induces phenotypic alterations in terms of adult hormonal axes and testicular cell kinetics as part of developmental plasticity

Bhavsar N.G[1], Lagu S.K[2] and Ramachandran A.V[3]*

[1]*Division of Anatomy and Physiology, Saint Louis community college, Saint Louis MO-63135-1408*
[2]*Department of Biology, Concordia University, 7141-Sherbrooke St. W, Montreal, QC Canada H.4.V.1R6*
[3]*Division of Reproductive Endocrinology, Department of Zoology, Faculty of Science, The M. S. University of Baroda, Vadodara 390002, Gujarat, India*

ABSTRACT

Time dependent glucocorticoid exposure in the neonatal period (21 days) and adult plasticity in terms of testes growth and function and neuro-endocrino axes have been studied in Charles foster rats. Adult hormonal profile and germ cell kinetics by histometric enumeration were evaluated. The results clearly suggest that, neonatal hypercorticolism induces adult plasticity in terms of advanced puberty onset, early onset of spermatogenesis and permanently hyporegulates neuro endocrine adrenal axis and up regulates thyroid axis. The hypothalamo-hypophyseal-gonadal axis also seems to be down regulated. The overall germ cell number is increased though Sertoli cell number is reduced, suggesting reduced apoptosis and increased number of germ cells being supported by Sertoli cells. Time dependent effect of corticosterone is seen in the form of relatively higher loss of advanced germ cells and decreased tubular length, basement area and Sertoli cell number in morning Cort treated rats. It is hypothesized from the present observations that, neonatal Cort excess can decrease germ cell apoptosis by genetic programming of Sertoli cell secretion and other functions.

Key words: neonatal, Corticosterone, plasticity, hormonal axis, germ cell dynamics.

INTRODUCTION

Early developmental events involving stress, hormonal disturbances and dietary alterations are shown to alter developmental plasticity and predispose adult physiology towards better health or disease. Stress induced increase in glucocorticoids is shown to affect testicular function and steroidogenesis in the adult [1, 2, 3]. 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 [4]. Glucocorticoids are known to be crucial for the maturation of foetal organ systems [5]. However, exposure of foetus to glucocorticoids has been shown to retard growth and precipitate disease in the adult [6, 7, 8]. These observations have led to the premise that glucocorticoids are involved in the programming of postnatal development of various systems. Foetal exposure to glucocorticoids may also be detrimental for post natal reproductive development as shown by the delayed onset of puberty in the female offsprings of mothers subjected to stress [9] or treated with adreno-corticotrophic hormone (ACTH) during gestation [10]. On these lines, another study involving glucocorticoid excess or insufficiency induced by appropriate treatments in pregnant rats from day 13 to term (day 23) had recorded reduced off spring 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 [11]. 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 post natal life, an effect that is manifested within the normal physiological range of glucocorticoid concentration.

Such experimental findings raised a logical question of the possible influence of glucocorticoid excess during the postnatal period of development on adult reproductive functioning . Interest in understanding the implications of prenatal and postnatal environmental differences in terms of nutritional, hormonal and other aspects on long term adult physiology by acting on the window of developmental plasticity, has witnessed resurgence [12]. Such effects on adult phenotype are increasingly being realized to be due to enzymatic modification of gene expression. 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 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 (T4), luteinizing hormone (LH) and testosterone (T) have also been evaluated. Further, quantitative histometry of the testis was also carried out to assess cellular dynamics and its impact on adult spermatogenesis.

MATERIALS AND METHODS

Animals and maintenance

Healthy male albino rats (Charles Foster strain) used for the present study were maintained in the animal house under a constant temperature range of 21-23°C and a lighting regimen of LD 10:14 throughout the period of study as per the CPCSEA guidelines and clearance by the ethical committee (Approval No. 827/ac/04/CPCSEA). The animals were fed with standard pelleted food (Lipton Rat Feed) and provided with water *ad libitum*. When the mated females delivered pups, males and females were separated and equal number of males were assigned to lactating mothers. The treatment was started on day 0 post partum and continued till 21 days (Weaning).

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 intraperitoneally (ip) with vehicle (0.9% saline) in the morning (0800 hrs) from day 0 to day 21 (CM).
(iii) Injected intraperitoneally (ip) with vehicle (0.9% saline) in the evening (1600 hrs) from day 0 to day 21 (CE).

Group II (Corticosterone treated) (Cort):
Newborn rat pups were injected I.P with Corticosterone 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:
The treatment was discontinued from day 22 and the animals were sacrificed at 35, 45, 60 and 90 days of age and, various morphometric, gravimetric and histocytometric studies were carried out. Fifteen day old animals were sacrificed for serum collection during the treatment period. The animals were killed under mild anesthesia and blood was collected by brachial venipuncture in ependorff tubes. They were centrifuged at 4000 rpm and serum was collected and stored at -4°C. Later, these serum samples were utilized for assay of various hormones. The viscera was cut open and testes were excised, blotted free of tissue fluids and weighed. The testes were then fixed in Bouin`s fluid and processed for paraffin wax histology.

Histology and histometry:
Testis was fixed immediately in Bouin`s fluid and processed for histological studies. Paraffin sections of 5 µ thickness were cut on a microtome and stained with Haematoxylin- Eosin (HE). For morphometry and enumeration of seminiferous tubules, homologous cross-sections from the middle part of testis showing the largest cross-sectional diameters were chosen. The diameter of seminiferous tubules and germinal epithelial thickness were measured using an ocular meter. The total Sertoli and germ cell number were estimated using morphometric methods based on the count of round objects in sections of known thickness [13] as modified from the reports of Wing and Christensen [14] and Russell *et al*.[15]. Inherent error was corrected using Floderus equation [16].

Hormone assay:
The blood for hormone assays was collected from the brachial vein under mild anesthesia before sacrificing the animals. T_3 and T_4 were assayed by ELISA kit purchased from Glaxo (product code H-T_3H-0010 and H-T_4H-0010) and expressed in ng/ml of serum. TSH, LH, Corticosterone

(Cort) and Testosterone (T) were assayed by RIA using kits obtained from Amersham International PIc.

Puberty Onset:
It was assessed by the capacity for preputial separation, determined by manual retraction of prepuce [17].

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

RESULTS

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

Postnatal growth:
The body weight of the experimental groups (CM and CE) was significantly less during the treatment period (Table 1). 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 weights of testes which tended to remain lower till 60 days in the experimental groups, became higher at 90 days though, statistically insignificant (Table 1).

Histology and Histometry:
Control testis revealed 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 both CM and CE animals showed increased number of compactly packed germ cells but 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 2). In fact, the number of Sertoli cell showed a significant decrement, more with the morning regimen. The tubular length was significantly decreased in CM rats and the total membrane area was decreased 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, but lesser than in the controls (2% in CE, 7% in CM and 10% in C) (Table 3) (Plates II, III, IV, V).

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) (Fig 1).
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 (Figs 4, 5, 6).

LH and Testosterone

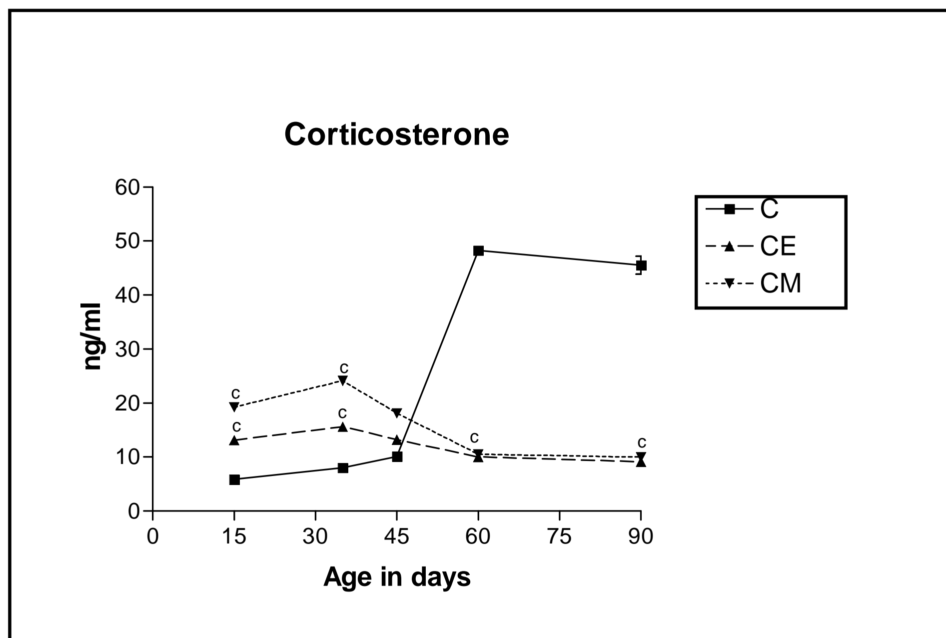
LH and T levels were significantly higher during the treatment period and this trend persisted till 35 days. Thereafter the levels of LH and T remained consistently and significantly lower in the experimental as compared to controls (Fig 2, 3).

Table I: Chronological alterations in body weight (g) and relative testis weight (g/100g) in control and Corticosterone treated rats

GROUPS	BODY WEIGHT					RELATIVE TESTIS WEIGHT				
	Age in days					Age in days				
	15	35	45	60	90	15	35	45	60	90
C	32.640 ±1.754	93.6 ±2.95	122.5 ±5.15	200.6 ±2.172	349.1 ±7.9	0.983 ±0.071	1.002 ±0.063	1.146 ±0.047	1.235 ±0.051	0.954 ±0.044
CE	24.87 ±2.72[a]	109.16 ±4.053[a]	160.83 ±3.43[c]	250.5 ±4.95[c]	332.66 ±10.58	0.895 ±0.048	0.904 ±0.044	1.128 ±0.057	0.921 ±0.089[c]	0.967 ±0.02
CM	26.12 ±2.54[a]	126.33 ±3.57[c]	150.833 ±3.380[c]	234.66 ±5.536[c]	333.76 ±6.49	0.967 ±0.050	0.98 ±0.041	1.09 ±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 are expressed as Mean±SEM of six animals.
[a] $p<0.05$, [c] $p<0.0005$

Fig 1: Serum corticosterone level (ng/ml) in control and corticosterone treated rats at 90 days of age



C- Control, CE-Low dose Corticosterone evening injection, CM-Low dose Corticosterone morning injection
Values are expressed as Mean±SEM of fifteen observations.
[c] $p<0.0005$

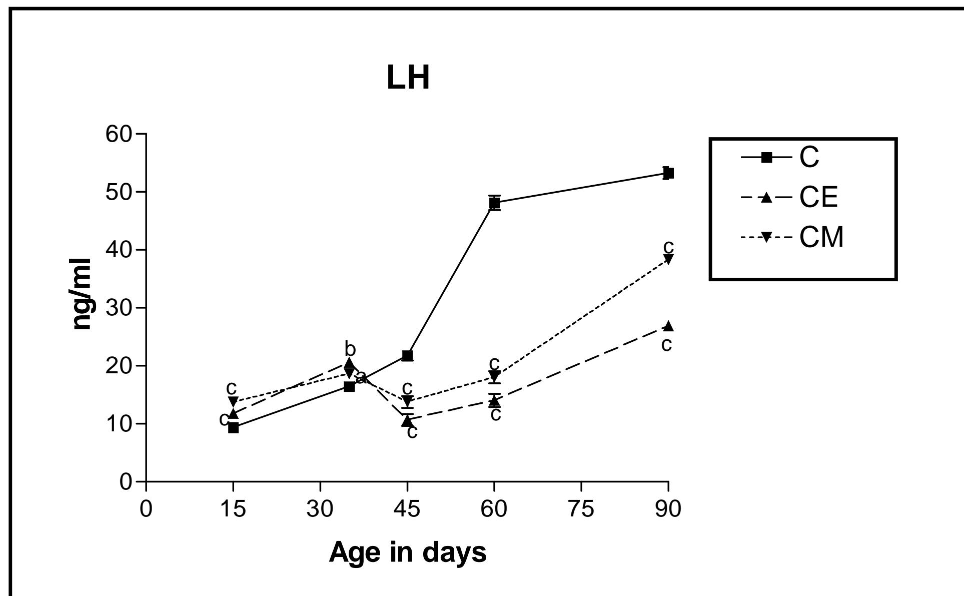
Table 2: Histometric enumeration of seminiferous tubules of control and Corticosterone treated 90 days rats

GROUPS	T_v In cc	S_D In cm	GE In cm	S_V In cm	S_L In cm	bm in cm^2	SC_N x 10^6	TGC_T x 10^6	AGC_T x 10^6	TGC_M x 10^6	AGC_M x 10^6	% LOSS
C	1.503 ±0.030	0.0279 ±0.0006	0.0074 ±0.0003	1.427 ±0.050	2321.03 ±94.20	204.045 ±5.23	32.49 ±1.80	311 ±6.30	280.84 ±5.60	13.39 ±0.26	12.1 ±0.15	10.00 ±0.0002
CE	1.775 ±0.16	0.031 ±0.0007 [b]	0.01 ±0.0002 [c]	1.668 ±0.15	2214.16 ±95.2	215.44 ±3.25 [a]	30.998 ±1.5	416.00 ±4.2 [c]	407.2 4±6.8 [c]	18.7 8±0.30 [c]	18.39 ±0.35 [c]	2.07 ±0.30 [c]
CM	1.47 ±0.05	0.034 ±0.0005 [c]	0.011 ±0.0003 [c]	1.398 ±0.040	1553.68 ±65.10 [c]	165.208 ±3.65 [c]	22.02 ±1.6 [c]	342.0 ±8.6 [b]	318.0 ±8.9 [b]	22.02 ±0.36 [c]	20.47 ±0.98 [c]	7.03 ±1.79

C- Control, CE-Low dose Corticosterone evening injection, CM-Low dose Corticosterone morning injection
Values are expressed as Mean±SEM of fifteen observations.
[a] $p<0.05$, [b] $p<0.005$, [c] $p<0.0005$

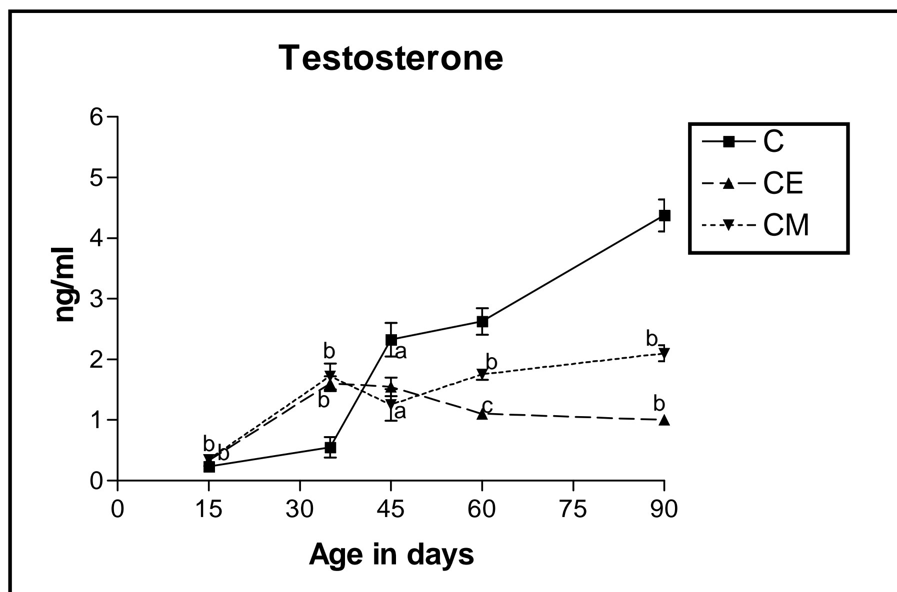
T_v – Volume of testis, S_D- Seminiferous tubule diameter, GE- Germinal epithelium thickness, S_v- Volume of seminiferous tubule, S_L- Length of seminiferous tubule, bm- basement membrane area of the seminiferous tubule, SC_N- Total Sertoli cell number in testis, TGC_T- Theoretical germ cell number per 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.

Fig 2: Serum LH level (ng/ml) in control and corticosterone treated rats at 90 days of age



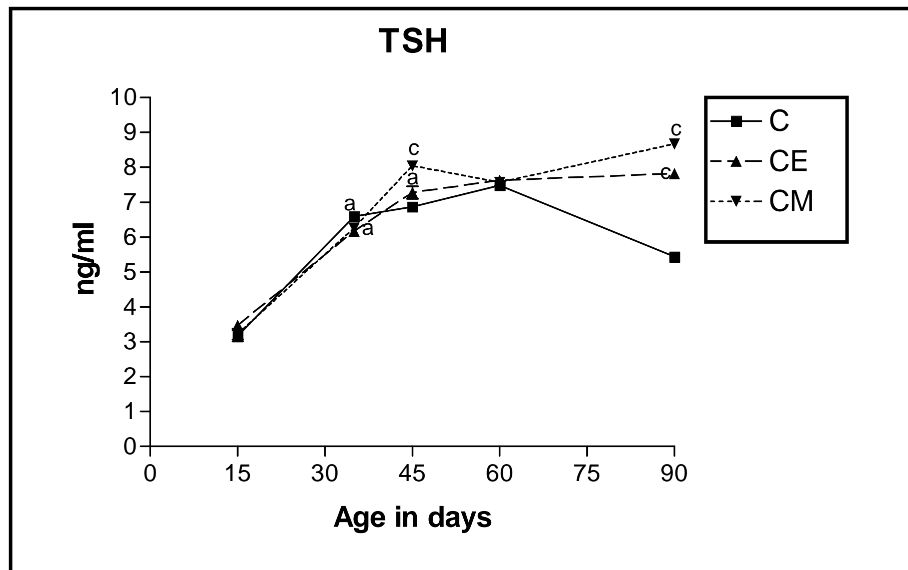
C- Control, CE-Low dose Corticosterone evening injection, CM-Low dose Corticosterone morning injection
Values are expressed as Mean±SEM of fifteen observations.
[b] p<0.005, [c] p<0.0005

Fig 3: Serum T level (ng/ml) in control and corticosterone treated rats at 90 days of age.



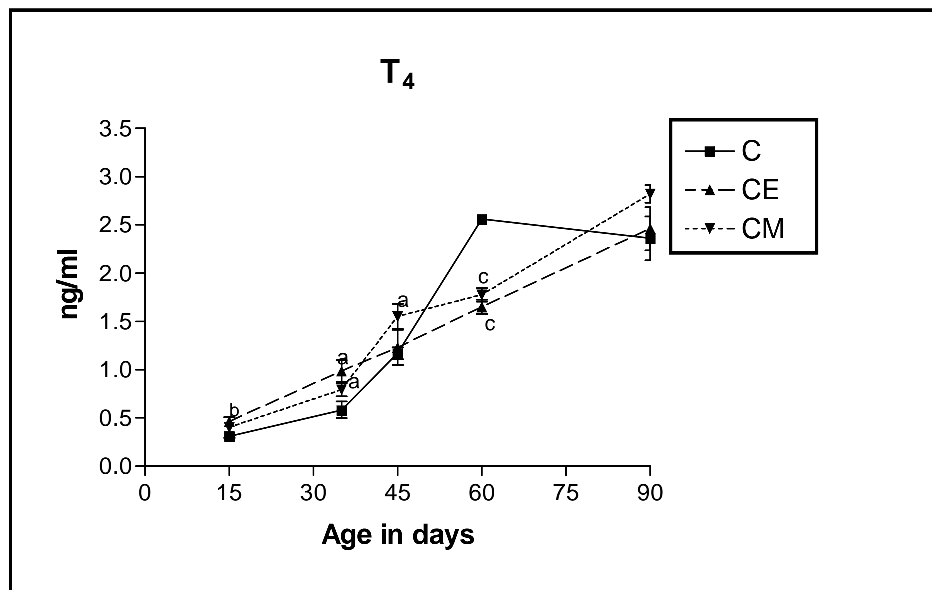
C- Control, CE-Low dose Corticosterone evening injection, CM-Low dose Corticosterone morning injection
Values are expressed as Mean±SEM of fifteen observations.
[a] p<0.05, [b] p<0.005

Fig 4: Serum TSH (ng/ml) in control and corticosterone treated rats at 90 days of age.



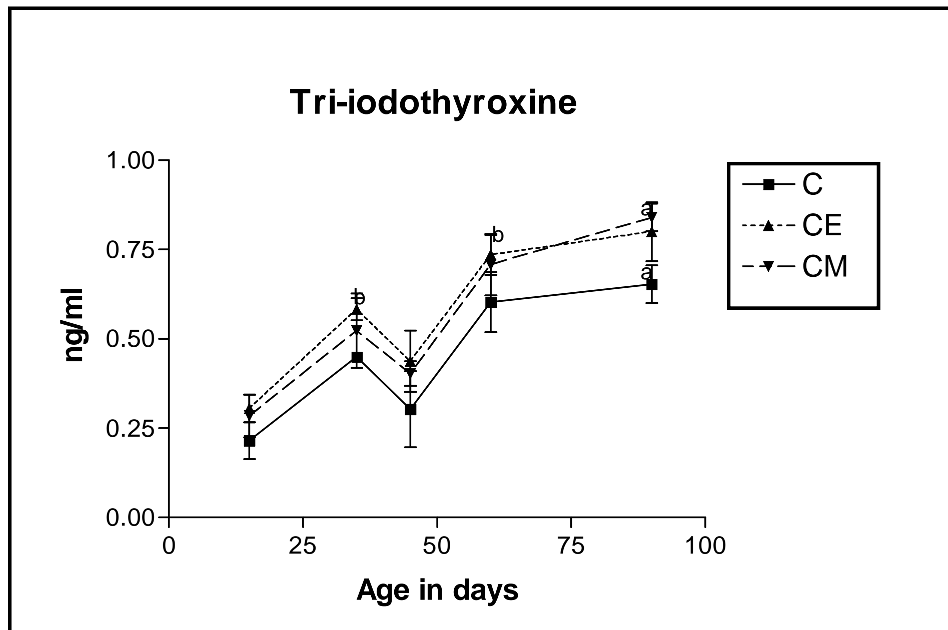
C- Control, CE-Low dose Corticosterone evening injection, CM-Low dose Corticosterone morning injection
Values are expressed as Mean±SEM of fifteen observations.
[a] $p<0.05$, [c] $p<0.0005$

Fig 5: Serum T_4 level (ng/ml) in control and corticosterone treated rats at 90 days of age



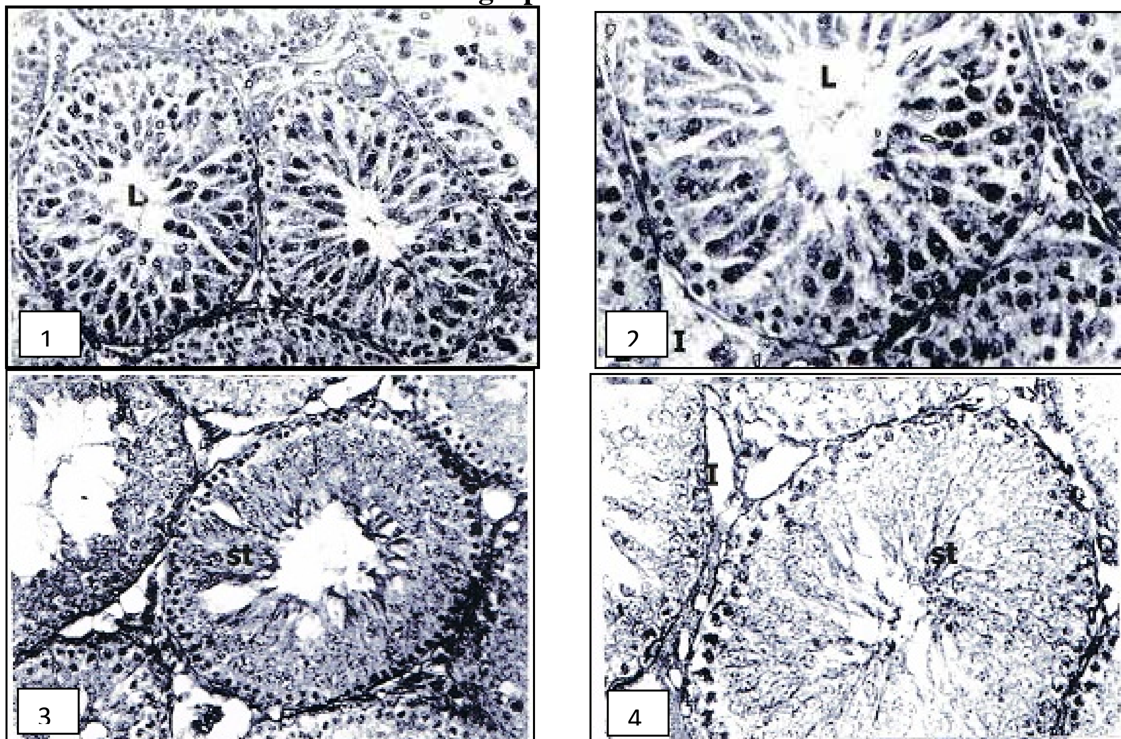
C- Control, CE-Low dose Corticosterone evening injection, CM-Low dose Corticosterone morning injection
Values are expressed as Mean±SEM of fifteen observations.
[a] $p<0.05$, [b] $p<0.005$, [c] $p<0.0005$

Fig 6: Serum T_3 level (ng/ml) in control and corticosterone treated rats at 90 days of age.



C- Control, CE-Low dose Corticosterone evening injection, CM-Low dose Corticosterone morning injection
Values are expressed as Mean±SEM of fifteen observations.
[a] p<0.05, [b] p<0.005

Plate I : Photomicrographs of section of testis of control rats.



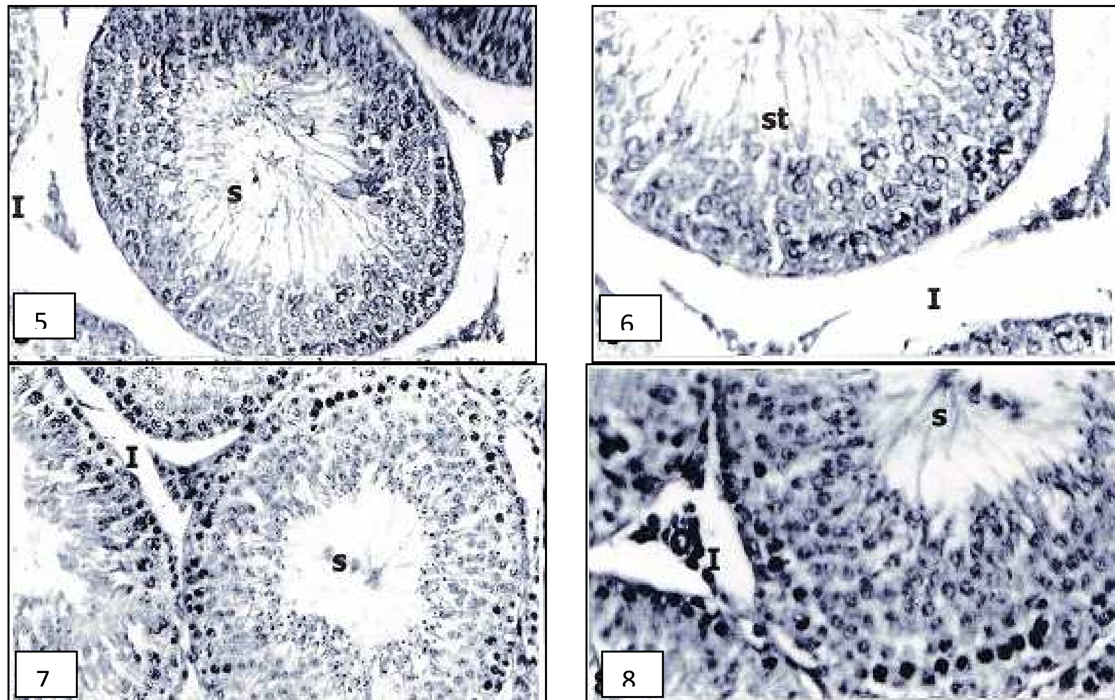


Plate I : Photomicrographs of section of testis of control rats.

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

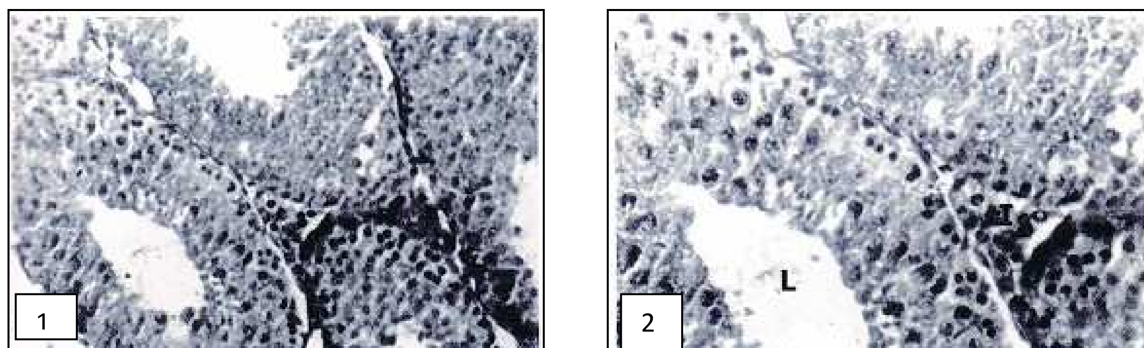
Figures 3 and 4 : *Sections of testis of 45 day of showing advanced stages of spermatogenesis and appearance of sperms in few tubules.*

Figures 5 and 6*: Sections of testis of 60 day old rats showing well established spermatogenesis and sperm in lumen.*

Figures 7 and 8: *Sections of testis of 90 day old rats showing prominent interstitium with well established spermatogenesis.*

Figures: 1, 3, 5 and 7 – 250x
Figures: 2, 4, 6 and 8-400x
Abbreviations :
I-Interstitium, L-Lumen, st-spermatids, S-sperm, D-Degeneration, rs- round spermatids.

Plate II : Photomicrographs of section of testis of rats treated with corticosterone postnatally.



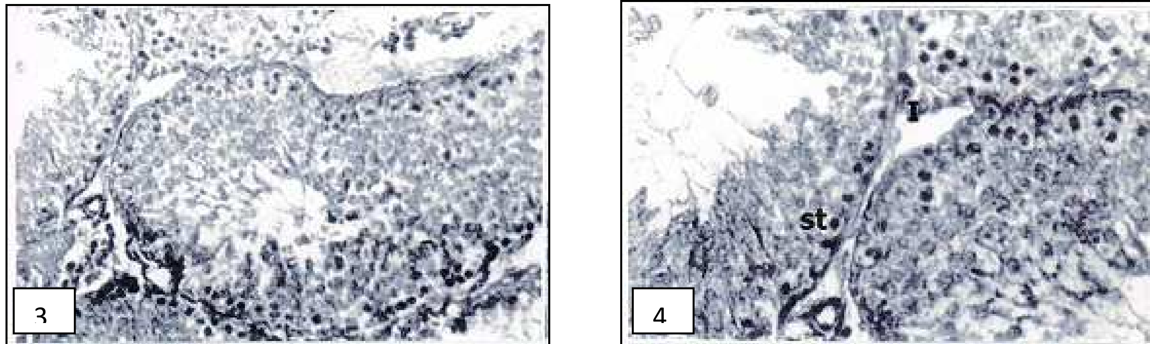
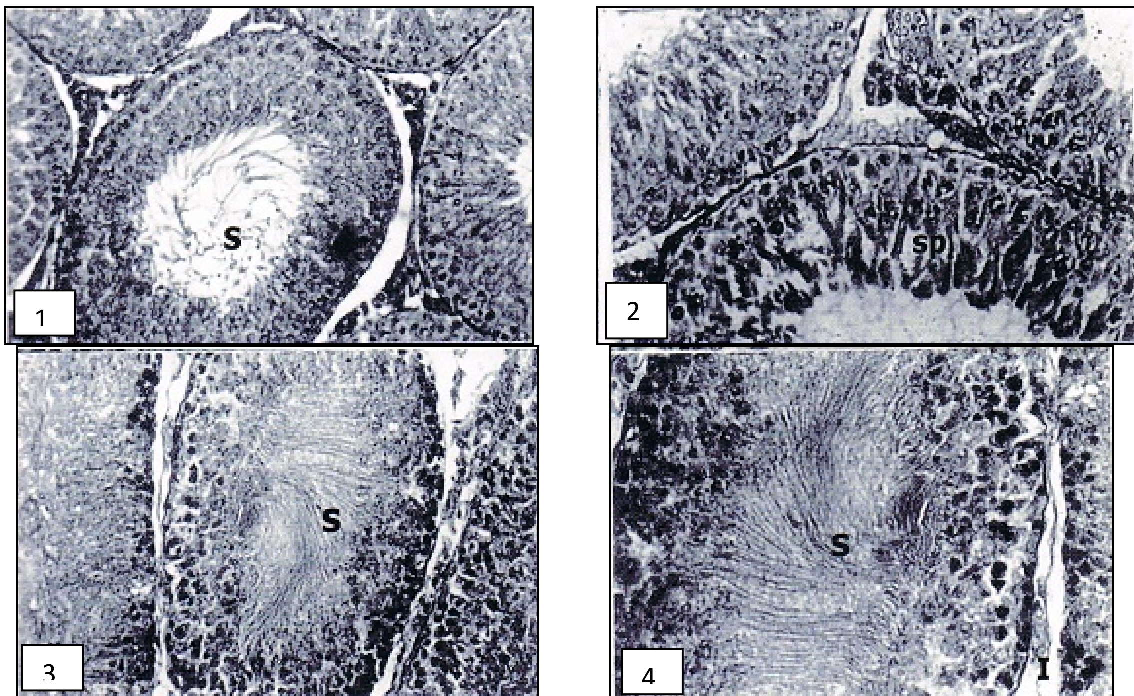


Plate II : Photomicrographs of section of testis of rats treated with corticosterone postnatally.

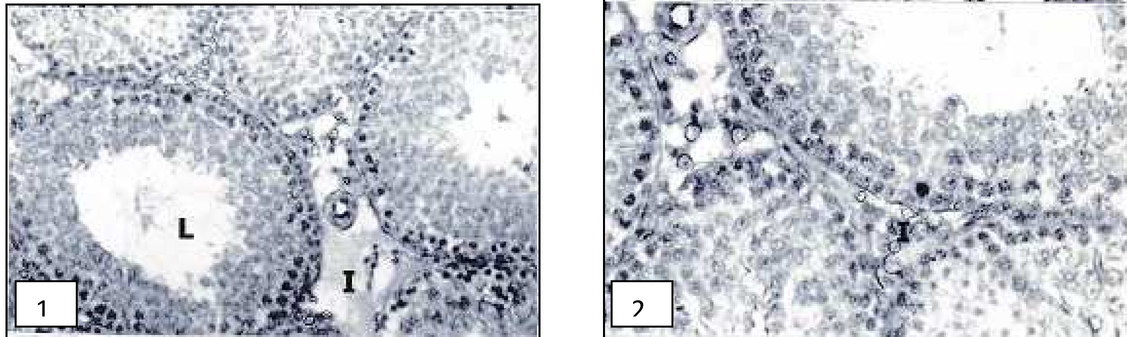
Figures 1 and 2 *: Sections of testis of 35 day old CE rats showing inhibited completion of meiosis*
Figures 3 and 4: *Testis section of 45 day old CE rats showing, fully established spermatogenesis and thinner population of sperms and more number of germ cells.*
CE- Low evening Corticosterone injection
Figures: 1 and 3- 250X
Figures: 2 and 4- 400X

Plate III : Photomicrographs of section of testis of rats treated with corticosterone postnatally



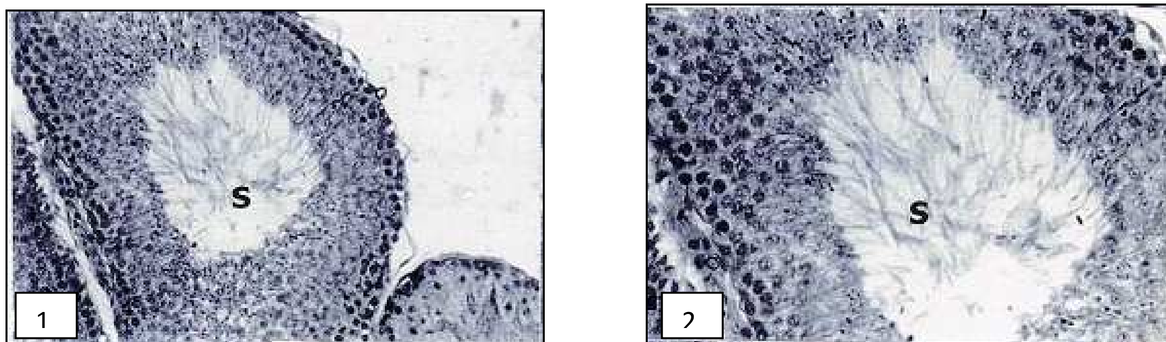
Figures 1 and 2*: Testis sections of 60 day old CE rats showing sperms and more number of germ cells.*
Figures 3 and 4: *Testis sections of 90 day old CE rats showing, high content of sperms and less number of germ cells. Prominent interstitium with large number of cells but smaller in size can be seen.*
CE- Low evening Corticosterone injection
Figures: 1 and 3- 250X; Figures: 2 and 4- 400X

Plate IV : Photomicrographs of section of testis of rats treated with corticosterone postnatally.



Figures 1 and 2 *: Sections of testis of 45 day old CM rats showing sperms and more number of germ cells.*
CM – Low morning Corticosterone injection
Figures: 1 and 2- 400X

Plate V: Photomicrographs of section of testis of rats treated with corticosterone postnatally



Figures 1 and 2 *: Sections of testis of 60 day old CM rats showing large number of germ cells.*
CM – Low morning Corticosterone injection
Figures: 1- 250X
Figures: 2- 400X

Discussion

The present investigation indicates that postnatal corticoid excess can affect the subsequent timing of puberty and qualitative and quantitative aspects of spermatogenesis in the adult condition. Though deleterious effect of excess corticosterone in adults has been documented [6, 7, 8], no report is available with regard to neonatal corticosterone excess on puberty onset or adult 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 [18], 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 is 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. These aspects are in contrast to the observed birth weight and attainment of puberty in rats due to induced corticosterone excess or deficiency in the foetal period, with the former resulting in decreased birth weight and delayed onset of puberty and latter in increased birth weight and earlier onset of puberty [6, 8, 11, 19]. Apparently, developmental plasticity effects of perturbations in glucocorticoids in prepubertal and postnatal periods are differential.

It is clear from the data 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, is marked by significantly pronounced rates in the CE and CM group of animals. This temporally hastened growth manifestation is also clearly reflected in the testes growth between days 0 to 45 (0.03N v/s 0.04 CE and 0.036CM). Much of this growth occurs between 35 and 45 days in CE and between 45 and 60 days in CM. The positive influence of transient neonatal exposure to corticosterone also manifests in the form of early attainment of puberty (42 days in CM and CE v/s 50 days in control).

The temporally enhanced growth dynamics is further confirmed by the advanced onset of spermatogenesis and appearance of sperm 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 are also marked by significantly greater 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 than morning exposure. Despite reduced tubular length and reduced Sertoli cell number, the significantly greater number of germ cells (both theoretical and actual) indicates attenuated germ cell loss by apoptosis, more so with the evening schedule. 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 is 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, synchronizes initiation of spermatogenesis for longer stretches in the tubule whish should, as a consequence, reduce the number of waves per tubule. The 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 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 [20]. 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 elevated influence on the hypothalamo-hypophyseal- thyroid (HHT) axis can also be inferred by the recorded higher circulating levels of T_3, T_4 and TSH. Previous studies on altered neonatal thyroid hormone status during the preweanling period have clearly established the role of T_3 in inducing Setoli cell differentiation and conversely, prolonged Sertoli cell proliferation and consequent increase in number due to hypothyroidism [21, 22, 23, 24, 25]. 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 sperm 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 sufficiently longer period seems to have a dampening influence on the hypothalamo-hypophyseal-gonad (HHG) axis. It is possible that, 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 corticoid exposure is tenable in the context of reported actions of foetal glucocorticoid exposure on the HHG axis [11] as well as on the HHA axis [26]. Since glucocorticoid receptors have been shown in the neural tissue [27, 28], pituitary gonadotrophs [29], ovary [30] and testis [31], 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 subjected to neonatal hypothyroidism [23, 25]. 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 [32, 33, 34]. 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 metabolizing enzyme of corticosterone) in the preweanling period [35] which could result in hyper stimulation and thereby altered Sertoli cell expression. Though glucocorticoids have been known to promote apoptosis in many tissues [36, 37, 38], the presently revealed antiapoptosis action in the testis is validated by the observation of an inhibitory role of glucocorticoids on apoptosis in neutrophils leading to increased neutrophil survival [39] and also in glomerular endothelial cells [40].

Developmental plasticity affecting different physiological and pathological aspects due to pre-natal, perinatal or postnatal programming due to exposure to hormones of stress is being increasingly realized in recent times [41, 42, 43]. Specially, exposure to corticoids or other stressors has been shown to induce adult hyper responsiveness of the HPA axis [44, 45, 46], disrupt the hypothalamus-hypophyseal-lactotroph axis [47], decrease adult Sertoli cell number [48], and downregulate HHG axis [49, 50].
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.

Acknowledgments
Thanks are due to Prof.M.M.Aruldhas, Department of Endocrinology, Dr. ALMPGIBMS, Taramani Campus,Chennai, for his kind help in hormone assay.

REFERENCES

[1] T.E.Orr, M.F.Taylor, A.K.Bhattacharya, D.C.Collins, D.R.Mann. *J Androl* **1994**, 15, 302-308.
[2] D.Maric, T. Kostic, R. Kovacevic. *J Steroid Biochem Mol Biol* **1996**, 58, 351-355.
[3] R.T.Chatterton, K.M.Vogelsong, Y.C.Lu, G.A.Hudgens. *J Clin Endocrinol Metab* **1997**, 82, 2503-2509.
[4] D.J.P.Barker. *BMJ publication group*. **1994**.
[5] J.D.Baxter, G.G.Rousseau. *Adv Exp Med Biol* **1979**, 1-24.
[6] R.Benediktson, R.S.Lindsay, J.Noble, J.Seckl, C.R.W.Edwards. *Lancet* **1993**, 341, 339-341.
[7] N.S.Levitt, R.S.Lindsay, M.C.Holmes, J.R.Seckl. *Endocrinol* **1996,** 64, 412-418.
[8] R.S.Lindsay, R.M.Lindsay, C.R.Edwards, J.R.Seckl *Hypertension* **1996**, 27, 1200-1204.
[9] J.A.Politch, L.R.Herrenkohl. *Physiol Behav* **1984**, 32, 95-99.
[10] P.W.Harvey, P.F.Chevins. *Teratology* **1987**, 35, 229-238.
[11] J.T.Smith, B.J.Waddell. *Endocrinol* **2000**, 141, 2422-2428.
[12] C.W. Kuzawa, E.A. Quinn. *Annu. Rev. Anthropol* **2009**, 38, 131–47.
[13] A.V.Ramachandran, N.G.Bhavsar, S.K.Lagu. *J.Anim.Morphol.Physiol.* **2001**, 48, 121-122.
[14] T.Y.Wing, A.K.Christensen. *Am.J.Anat* **1982**, 165(1), 13-25.
[15] L.D.Russel, R.A.Etllin, A.P.Sinha-Hikm, E.D.Clegg. Catche River Press, **1990**, Clear water.
[16] S.Floderus. 1994: As cited by Yangzhenqwei, Wreford N G, David M. de Krester. *Biol Reprod* **1990**, 3, 629–35.
[17] C.C. Korenbrot, I.T.Huhtaniemi, R.I.Weiner. *Biol Reprod* **1977**, 17, 298-303.

[18] R.E.Poland, M.E.Weichsel, R.T.Rubin. *Endocrinol* **1981**, 108, 1049-1054.
[19] P.J.Burton, B.J.Waddell. *Biol.Reprod* **1999**, 60, 234-240.
[20] D. Suchecki, S.Tufik. *Am.J.Physiol* **1997**, 99, R1332-1338.
[21] R.A.Hess, P.S.Cooke, D.Bunick,J.D.Kirby. *Endocrinol* **1993**, 132, 2607-2613.
[22] L.M. van Haaster, F.M. de Jong, R. Docter, D.G. de Rooij. *Endocrinol* **1992**, 133, 755-760.
[23] P.S.Cooke, R.A.Hess, J.Porcelli, E. Meisami. *Endocrinology* **1991**, 129, 244–8.
[24] L.R. De Franca,R.A.Hess,P.S.Cooke,L.D.Russell. *Anatomic Res* **1995**, 242, 57-69.
[25] S. K. Lagu, N. G. Bhavsar, R. K. Sharma, A. V. Ramachandran. *Neuroendocrinology Letters* **2005**, 26(6),780–788.
[26] M.J.Meaney, V.Viau, S.Bhatnagar, K.Betito, L.Iny, D.O`Donnell. *J.Steroid Biochem.Mol.Biol*. **1991,** 39, 265-274.
[27] L.P.Reagan, B.S.McEwen. *J Chem Neuronant* **1997**, 13, 149-167.
[28] S.Daikoku, I.Koide. *J Comp Neurol* **1984**, 393, 34-47.
[29] S.M.Kilen, M.Szabo, G.A.Strasser, J.M.McAndrews, S.J.Ringstrom, N.B.Schwartz. *Endocrinol* **1996**, 137, 3802-3807.
[30] A.E.Michael, L.A.Pester, P.Curtis, R.W.Shaw, C.R.Edwards, B.A.Cooke. *Clin Endocrinol* **1993**, 38, 641-646.
[31] C.Monder, Y.Miroff, A.Marandi, M.P.Hardy. *Endocrinol* **1994,** 134, 1199-1204.
[32] C.Huckins. *Anatomica rec* **1978**, 190, 905-926.
[33] D.G.deRooji, J.M.Janssen. *Anatomica rec* **1987**, 217, 124-130.
[34] J.Blanco-Rodriguez. *Int J Androl* **1998**, 21, 236-248.
[35] M.P.Hardy. *In:Encycl Reprod* **1998**, 1021-1033.
[36] J.A.Gonzalo, A.Gonzalez-Gracia, C.Martinez, G.Kruemer. *J.Exp Med* **1993**, 177, 1239-1246.
[37] A.H.Hassan, P.Vonrosensteil, V.K.Patchev, F.Holsboer, O.F.Almeida. *Exp Neurol* **1996**, 140, 43-52.
[38] B.J.Waddell, S.Hisheh, A.M.Dharmarajan, P.J.Burton. Biol Reprod 2000, 63, 1913-1917.
[39] Cox G *J of Immunol* **1995**, 154, 4719-4725.
[40] U. K. Messmer, G. Winkel, V. A. Briner, J. Pfeilschifter. *Br J pharmacol* **1999**, 127,1633-1640.
[41] A.M.Dufty, J. Clobert, A.P. Moller. *Trends in Ecology and Evolution* **2002**, 17, 190-196.
[42] L.F.Abigail, G.A.Dino, F.J.Alison. *Physiology* **2006**, 21, 29-37.
[43] L.A.Zakharova. *Recent Patents on Endocrine, Metabolic & Immune Drug Discovery* **2009**, 3, 11-27.
[44] A.Yamazaki, Y.Ohtsuki, T.Yoshihara, S.Honma, K.Honma. *Physiology and Behavior* **2005**, 86, 136-144.
[45] E.Kanitz, W.Otten, M.Tuchscherer. *Journal of Endocrinolgy* **2006**, 191, 207-220.
[46] K.H.Poore, J.P.Boullin, J.K.Cleal, J.P.Newman, D.E.Noakes, M.A.Hanson, L.R.Green. *The Journal of Physiology* **2010**, 588, 2219-2237.
[47] S.McArthur, Z-L. Siddique, H.C.Chistian, G.Capone, E.Theogaraj, C.D.John, S.F.Smith, J.F.Morris, J.C.Buckingham, G.E.Gillies. *Endocrinology* **2006**, 147, 1904-1915.
[48] B.Kotsampasia, C.Balaskasa, G.Papadomichelakisb, S.E.Chadioa. *Animal Reproduction Science* **2009**, 114, 135-147.
[49] S.M.Rhind. *Animal Reproduction Science* **2004**, 82, 169-181.
[50] X.F.li, J.S.Kinsey-Jones, A.M.I.Know, X.Q. Wu, D.Tahsinsay, S.D.Brain, S.L.Lightman, K.T.O`Byrne. *Endocrinolgy* **2007**, 148, 5984-5990.