GENERAL CONCLUSIONS AND POST-SCRIPT

The present study was undertaken essentially to understand the impact of altered hormonal *milieu* in the neonatal stage (preweanling period) on growth and maturation of the testes and adult serum hormonal status as long-term consequences. In this context, experimentally induced disturbances in the adrenal and pineal hormone status have been used to understand the relative and interactive roles of these hormonal principles. The experiments were essentially carried out under a short photoperiod of LD 8:16 and a constant temperature regimen of 21°C ± 2°C. Individual effects of glucocorticoid excess or deficiency or melatonin excess, as well as melatonin excess in combination with hypo or hypercorticalism have all been assessed within the framework of the above cited objectives.

Corticosterone excess or insufficiency has been reported to show detrimental effects on male gonadal functions, especially steroidogenesis in rats and man. However, the present study clearly suggests that neonatal corticosterone excess in the physiological range can have favourable influences. In this respect a clear difference in terms of dosage and time of corticosterone administration has been realised. Smaller doses of

corticosterone resulting in milder hypercorticalism not only hastens puberty, but also augments spermatogenesis. Again the evening exposure seems relatively more favourable. The influences recorded are:

1. Hastened body and testes growth dynamics.
2. Early puberty attainment and advanced onset of spermatogenesis.
3. Higher number of germ cells with dense population of spermatozoa, more prominently in the evening schedule.
4. Reduced tubular length and Sertoli cell number but significantly increased number of germ cells.
5. Relatively more germ cell loss in the morning schedule.
6. Number of germ cells per meter length of tubule is more in the morning schedule.
7. Faster growth of seminiferous tubule as 65% of the length is attained at 35 days itself.
8. Corticosterone treatment (especially evening treatment) shows only very few stages of spermatogenesis in the sections of the tubules.
9. Lowering of the central set point of HHA and HHG axes as long-term consequences while the HHT axis is elevated.
10. Though higher dose of corticosterone shows favourable influence on the number of germ cells, there is a perceptible deleterious effect on the survival of mature germ cells.

11. The higher dose of corticosterone shows a time dependent differential effect on the HHT axis with a permanently elevated set point with morning schedule and an opposite lowered set point with evening schedule.
12. The hypercortical (MET) animals also showed early onset of spermatogenesis and appearance of sperms.
13. There is increased germ cell loss and degeneration in MET animals.
14. There is significantly reduced number of germ cells per meter length of the tubule.
15. There is no effect on the HHA axis but the HHG axis is differentially affected as marked by higher levels of LH and lower levels of T.
16. The HHT axis is up regulated at the hypothalamo-hypophyseal level but the sensitivity of the thyroid to TSH is reduced.

These observations clearly bespeak of a favourable influence of optimum levels of corticosterone excess during the neonatal period on adult spermatogenic functions. The significantly increased germ cell number and sperm mass despite the reduced number of Sertoli cells is clearly due to reduced germ cell apoptosis and degenerative loss. The reduced apoptosis is hypothesized as a long-term effect of neonatal corticosterone. This effect (anti-apoptotic) is presumed to be mediated through a permanent genetic reprogramming of Sertoli cell secretory functions. This also results in an

increased capacity of the Sertoli cells for germ cell support. The purported anti-apoptotic effect of corticosterone is validated and strengthened by the observed higher germ cell degeneration and loss in the testis sections of rats rendered neonatally hypocorticalic. The neonatal corticosterone is also considered favourable for an overall body and testes growth. The observation of only a few stages of spermatogenesis in the sections of tubules leads to a speculation that corticosterone might exert a synchronizing effect on initiation of spermatogenesis for longer stretches of the tubule which as a consequence should theoretically reduce the number of waves per tubule. It is also clear that corticosterone excess in the neonatal phase can have permanent effects on the set points of adrenal, thyroid and gonad axes.

These studies have thrown up certain intriguing questions, which need to be addressed at length for getting relevant answers. Some of these points, which need future substantiation and validation are:

1. The role of neonatal corticosterone as an augmenter for reproductive growth and, its role in Sertoli cell genetic programming and expression of genes regulating apoptosis.
2. Its role in synchronization of initiation of spermatogenesis over long stretches of seminiferous tubule and thereby reducing the number of waves.
3. Its modulatory influence on various hormonal axes and consequent readjustments of the set points.

Concurrent to studies on neonatal corticosterone alterations, the effect of neonatal pineal hormonal excess (hypermelatonemia) was also investigated. Subsequently the same was studied in combination with simultaneous hyper/hypocorticalism. These studies have shown the following key observations:

1. Melatonin (MT) increases body weight.
2. However the relative weight of testes is reduced.
3. The tubular length is decreased but the diameter and germinal epithelial thickness are increased.
4. The number of germ cells is significantly increased but there is marked loss of advanced germ cells by sloughing.
5. MT treatment permanently lowers the HHG axis but elevates the HHT axis though with reduced T_4 levels.
6. The combination of evening corticosterone and melatonin (CE+MT) shows body growth changes intermediate to CE and MT animals and testes growth changes similar to MT.
7. The relative weight of testes is also intermediate to that of CE and MT animals.
8. Higher degree of germ cell loss with premature detachment of spermatids and spermatozoa is a feature.

9. Differential effects on the HHG axis is marked by higher LH level and lower T level.
10. The HHA axis is permanently down regulated.
11. The premature detachment of advanced germ cells is also found in CM+MT animals.
12. CM+MT animals also show very poor germ cell mass.
13. The tubular length, basement membrane area and total Sertoli cell number are reduced as in MT animals.
14. The germ cell number is also significantly reduced with higher degree of loss.
15. The HHG and HHA axes are permanently down regulated in CM+MT rats but HHT axis is differentially altered with higher TSH level and lower T_3 and T_4 levels.
16. The MET+MT animals show body growth rate similar to MET animals. But the testes growth rate and relative growth rate are similar to that of MT animals.
17. The MET+MT animals show more potentiated MT effects on decrement in tubular length, volume, basement membrane area and Sertoli cell number.
18. The number of germ cells, which is significantly reduced in MET animals is nullified by simultaneous MT treatments.

19. The degree of germ cell loss is decreased compared to that of MET animals.
20. The characteristic MT induced degeneration of germ cells is nullified by MET treatment.
21. The HHA axis is reset at a lower level.
22. The HHT axis is differentially affected marked by higher TSH level and lower T_3 level.
23. The HHG axis is also down regulated.

The generalized conclusions that could be drawn from the above are that neonatal melatonin augments body growth, probably by increasing growth hormone (GH) secretion or by potentiating its action. The increased tubular diameter and germinal epithelial thickness despite the reduced tubular length is essentially due to increased germ cell number primarily due to decreased germ cell apoptosis: a corticosterone effect as circulating corticosterone level is elevated during MT treatment as well as in the immediate post-treatment period. Paradoxically MT also induces great degree of germ cell degeneration but this is far outweighed by the decreased apoptosis *per se*. The MT animals also show faster tubular growth, which is either a direct effect or an indirect one mediated through corticosterone. The loss of advanced germ cells by premature detachment signifies a probable long-term effect of neonatal MT on Sertoli cell/ germ cell adhesive properties.

Though the lowering of the set point of HHT axis is akin to what is seen in corticosterone treated rats, the reduced T_4 level, a novel feature, is probably due to MT induced altered T_4 : T_3 secretory ratio or a case of increased peripheral conversion of T_4 to T_3. MT directly or indirectly through corticosterone has a definite dampening influence on the HHG axis.

The CE+MT combination has complex interactive influence on body and testes growth. In a combination of evening hypercorticalism and hypermelatonemia, the favourable influence of corticosterone on spermatogenesis gets not only totally nullified but the deleterious effects of MT on advanced germ cells gets further potentiated. This alludes to a possible higher expressivity of melatonin on a corticosteroid background or put in another way it suggests that corticosterone has potentiating effect on melatonin effect on, germ cells.

It is likely that CE+MT combination in the neonatal period either can up regulate the GnRH pulse regulator and/or up regulate the sensitivity of pituitary gonadotrophs. It is also likely that the increased LH secretion is due to decreased sensitivity of the GnRH neurons and/or the pituitary gonadotrophs to the negative feedback action of testosterone. Concurrently, the reduced T secretion also attests to a probable reduced sensitivity of pubertal and post pubertal Leydig cells to LH. The permanent lowering of HHA axis could be a direct action of MT or even an indirect one mediated through corticosterone.

The potentiated damaging influence of MT on advanced germ cells in presence of corticosterone is apparently time independent as even a combination of CM+MT depicted the same degree of damage. It is also likely that CM+MT combination has some detrimental effect on spermiogenesis per se.

Though there is time independent influence of corticosterone in potentiating the harmful effect of MT on germ cells, CM seems to be more vulnerable in this respect. The lowering of the set points of HHG and HHA axes is probably a consequential effect of the elevated corticosterone level. As MT rats does not show any alteration in the HHA axis, it is clear that the corticosterone effect on its own axis cannot be nullified by MT. The differential alteration of HHT axis could be by way of increased TRH secretion coupled with decreased sensitivity of thyroid.

The increased growth Kinetics of body and testis in the post treatment period in MET+ MT rats is primarily due to increased corticosterone level brought about by the rebound increase on lifting of adrenal suppression by MET.

The influence of melatonin on the growth kinetics of testis constituents is apparently more potentiated in a lower corticosterone background. MT seems to be capable of nullifying the negative influence of MET on germ cell number by way of increased apoptosis. A combination of melatonin excess and corticosterone is also capable of down regulating HHG axis. Differential

interaction of the two hormonal status affects the HHA axis differentially by increasing TRH secretion at the central level and decreasing T_3 output and/or reduced T_4-T_3 conversion at the peripheral level. The lowering of the set point of HHG axis as seen in MT rats is further potentiated by simultaneous hypocorticalism.

These conclusions drawn based on the effects manifested by hypermelatonemia in combination with hyper or hypocorticalism suggest definite intricate interactions between melatonin and glucocorticoids during the neonatal period in modulating adult reproductive functions. The inferred roles of corticosterone in preventing germ cell apoptosis, of melatonin in altering the Sertoli cell-germ cell interactions as well as the potentiating and nullifying effect of each other, all need to be evaluated in the future to validate the herein inferred functional roles or garbs.

Overall, it can be concluded that melatonin-glucocorticoid interactions in the neonatal period are of crucial significance in modulating the postnatal growth, maturation and functional expression of adult testis and accessory organs.