GENERAL CONSIDERATION AND POST-SCRIPT

Fetal and neonatal endocrine disruption or dysfunction is likely to have long-lasting effects manifested in the adults as altered physiological and endocrine functions and/or homeostasis. Apparently, late fetal and neonatal periods are imprinting phases. These permanent alterations in adult homeostasis can be considered as "organizational" effects of neonatal perturbations. It is to assess such organizational changes that studies were initiated in this laboratory involving neonatal hypermelatonemia, hypo or hyperthyroidism and hypo or hypercorticalism on adult testis functions and hormonal status. The present study was undertaken essentially as an extension of the above study to decipher the impact of neonatal light induced hypomelatonemia as well as its interaction with hypothyroidism and hypercorticalism as well as, melatonin replacement. Further, due to paucity of information and to have comparative sex biased effect if any, similar studies on manipulation of female neonates have been undertaken in terms of hypermelatonemia, hypothyroidism and combination of hypermelatonemia and hypothyroidism on adult ovarian histoarchitechture and hormone profiles.

The laboratory rat has not been found to be very photosensitive and at best strain dependent differential response have been recorded in recent times. However, in the present study, light induced functional pinealectomy, by exposure of male pups of Charles Foster strain of rats has shown subtle changes on adult testis histoarchitechture and on serum hormone titres. Though there is no significant effect either on body or testes weight, there is earlier onset of spermatogenesis by 45 days which is related with increased thyroid hormone levels in the prepubertal stages. It is now well established that prepubertal elevation in thyroid hormone levels induces Sertoli cell differentiation terminating the postnatal phase of Sertoli cell proliferation. Neonatal functional pinealectomy creating a hypomelatonemic state has an up regulating influence on the thyroid hormone axis as, T4 and T3 levels are permanently elevated. Since the elevation in T4 and T3 levels is occurring despite lowered TSH level, it is inferable that there is increased sensitivity of thyroid gland to TSH. A natural question that arises is whether the higher melatonin level in the normal neonates regulates thyroid hormone sensitivity to TSH?

The previously observed increased TSH level and decreased T4 level in neonatal hypermelatonemic rats is supportive of this inference (Ramachandran *et al.*, 2004). Another significant observation is the reduced number of germ cells. The increased

germ cell degeneration/ apoptosis of advanced germ cells like spermatids and sperms. This decreased germ cell number and increased loss of advanced cells are co-related with increased corticosterone (CORT) titres seen in the prepubertal periods due to LFPx. Apparently, high CORT levels in the prepubertal phases under a low melatonin background is detrimental to germ cell survival by promoting apoptosis as well as reduced adhesion between Sertoli cells and advanced germ cells. Supportive evidence for this conclusion is provided by the works of Yazawa et al. (1999, 2000) and of Biagini and Pich (2002). Neonatal up-regulation of HHG axis is marked by the increased LH and T levels. This is confirmed by the decreased LH and T levels observed in rats rendered neonatally hypermelatonemic.

A follow-up study conducted to see possible reversibility of LFPx induced effects by simultaneous melatonin treatment in the neonatal period; a decreased testes weight was recorded. Though the hypothalamo-hypophyseal-thyroid axis as well as initiation of spermatogenesis where more like LFPx rats (levels of hormones however, were relatively lower), there was a significant increase in seminiferous tubular length, basement membrane area and total number of germ cells. These changes are quite distinct to those observed in LFPx or melatonin alone rats. While, the survival of early germ cells is relatable with increased prepubertal CORT level

(as also inferred previously, Bhavsar, 2001), the loss of late germ cells is relatable with a combination of LFPx+MT and/or CORT. Melatonin administration not only reverses the LFPx induced up-regulation of HHG axis but also induces hypermelatonemic effect of decreasing the activity of HHG axis as seen previously (Lagu, 2001). These observations suggest the need to understand melatonin-corticosterone interactions and the ability of melatonin to influence the organization of the hypothalamo-hypophyseal-adrenal (HHA) and HHG axes to an adult pattern and level of homeostasis.

Since neonatal hypothyroidism has shown favourable influence on adult testis structure and functions and LFPx has revealed some adverse effects, an attempt was made to investigate the combined effect of LFPx and hypothyroidism an adult testis histoarchitechture and hormonal profiles. In terms of body and testes weights, the observed effect of reduced body weight and increased adult testes weight are more characteristic of HPOT effects rather than LFPx. Obviously on these aspects, HPOT has an over-riding influence over that of LFPx. Since the initiation and completion of spermatogenesis was delayed and there was increased tubular diameter, Sertoli and germ cells, it is clear that the influence of neonatal HPOT is more dominant and is essentially due to the prolonged phase of Sertoli cell proliferation postnatally

due to the low level of thyroid hormones which delays Sertoli cell differentiation and progression of spermatogenesis. A novel observation however was the more than doubled increase in tubular length seen in LFPx+HPOT animals. It is interesting that in an earlier study on surgical pinealectomy, an increase in tubular length was noted and was related with the increased TSH level in the neonatal period due to hypomelatonemia (Sharma, 1996) as neonatal increase in TSH has been shown to contribute to increase in tubular length. Apparently the light induced hypomelatonemia as well as, as yet other unknown pineal factors which are not reduced due to light seem to interact with HPOT status in bringing about an even greater increase in tubular length as seen in the present study. LFPx+HPOT combination also seems to down regulate the HHG axis seen by the lower LH and T levels. This down regulation of HHG axis is to a greater extent offset by an increase in Leydig cell number, which could also be related with a reduced sensitivity of these cells to LH. Similarly, there is also down-regulation of HHA axis and these effects on HHG and HHA axes are similar to those seen in HPOT rats (Lagu, 2001). However, the up regulation of HHT axis is quite distinct to that of HPOT effect and hence appears to be a combination effect of light and hypothyroidism.

A previous study on neonatal CORT administration mimicking a physiological state of stress was shown to have a time dependent effect of evening CORT increasing testes weight and both morning or evening treatment having a detrimental effect on the population of spermatids and sperms, more pronouncedly in the former. The present study on LFPx+CORT seems to have more potentiated effect on the apoptotic loss of late/mature classes of germ cells alluding to a combination effect. Such a combination effect potentitating the influence of CORT alone is further justified by the observed increase in early germ cells, a potentiated favourable anti-apoptotic influence of CORT on early germ cells. LFPx+CORT also seems to down regulate all the three endocrine axes i.e. HHG, HHT and HHA. The reduced serum level of T is corroborated by the increased titre of LH which in turn is related with the prominent Leydig cells observed. The decreased T level despite the prominent Leydig cells is suggestive of decreased sensitivity of the cells. The present observations have thrown up more avenues of future investigations in terms of alterations in the critical organizational neonatal phase, leading to long-term changes and affecting adult physiology and homeostasis with reference to many systems.

In the course of the present study, an attempt was also made to see the possible impact of neonatal manipulations in females as opposed to all the above and past studies on males. The first

paradigm that was evaluated was the influence of neonatal hypermelatonemia on adult ovarian histoarchitectural dynamics and hormone profiles. In terms of body weight, a clear cut sex difference due to neonatal MT treatment is evident as the treated females weighed lesser in adults compared to males in a previous study (Ramachandran *et al.,* 2004). This sex difference is attributed to the difference in the response of the growth hormone axis. The ovarian histoarchitechture has revealed a favourable influence of neonatal MT as there was a generalized increase in the number of all classes of follicles. This favourable influence seems to be more due to reduced apoptotic loss of follicles as revealed by the significantly reduced number of atretic follicles. A future line of investigation in this context should be to find out whether neonatal melatonin excess has a long lasting influence on the developing ovary in terms of genetic reprogramming leading to activation of follicle survival factor and/or inhibition of apoptotic factors. The increased estrogen:progesterone ratio as well as increased T3 and T4 levels even in the prepubertal period are favourable changes for follicular health. Apparently, neonatal hypermelatonemia has a long term favourable influence on follicular survival either by a direct action and/or an indirect action through thyroid hormones. To test this inferred role of thyroid hormone, the effect of neonatal hypothyroidism was evaluated. Confirmation of the above

inference was obtained as the neonatally hypothyroid rats showed more atretic follicles, decreased estrogen:progesterone ratio and ovary weight. Apparently neonatal hypothyroidism seems to have long-term effects on inducing follicular apoptosis. The present study clearly suggests the need for an optimum level of thyroid hormones in the neonatal period for normal ovarian follicular dynamics. Since HPOT and MT treatments had shown unfavourable and favourable effects respectively, the effect of a combination of HPOT+MT was tested. Interestingly, the combination of hypothyroidism and hypermelatonemia had a favourable influence on a quantitative basis as, increased numbers of pre-antral follicles were observed. However, this favourable influence on pre-antral follicles is offset by increased apoptotic loss of antral follicles as noted by increased atresia and reduced number of antral follicles and corpora lutea. This raises a question of whether there is a differential effect on apoptosis of pre-antral and antral follicles? And how could such differential effects be manifested and regulated on a long term basis by a combination of neonatal hypothyroidism and hypermelatonemia? It is also clear that the effect of HPOT on body and ovarian weight and, volume cannot be overcome by melatonin. The HPOT+MT status of neonates seems to also affect the endocrine profiles differentially as, there is reduction in estrogen and progesterone levels and increase

in T4 and T3 levels, suggesting a down regulation of HHG axis and up regulation of HHT axis.

It can be concluded from the present studies that neonatal hormonal perturbations have definite long lasting permanent effects on gonadal functions and hormonal profiles and further that, there is also a sex dependent differential effect. Though many explanations are forthcoming based on the observations made in the course of this study, many new avenues of investigation have been opened and new queries raised. Further searching investigations are needed to embellish the present observations on a firmer edifice.