CONCISE SUMMARY

Endocrine secretions in vertebrates represent highly potent and specialized organic molecules that control and regulate biological functions. The light/dark cycle, to which animals, including humans are subjected, has a profound influence on their physiology. On an annual basis, the seasonal variations in the day length and temperature differences influence reproduction, migration and hibernation. The processes of regulation of this endogenous rhythm, the pineal acts as an intermediary between the environment and the endocrine system and melatonin, as the chemical link. The vertebrate pineal gland produces and secretes melatonin following circadian rhythm with high levels at night and low levels during the day (Reiter, 1991 Krause and Dubocovich, 1990). During the last decade much attention has centered on melatonin, one of the hormones of the diffuse neuroendocrine system. For many years it was considered to be only a hormone of pineal gland. As soon as highly sensitive antibodies to indole-alkylamines became available, melatonin was identified not only in the pineal gland but also in extra pineal tissues. These included retina, harderian gland, gut mucosa, cerebellum, airway epithelium, liver, kidney, adrenals, thymus, thyroid, pancreas, ovary, carotid body, placenta and endometrium. It has also been localized in non-neuroendocrine cells such as mast cells, natural

killer cells, eosinophilic leukocytes, platelets and endothelial cells. These lists of cells indicate that melatonin has a unique position among the hormones of the diffuse neuroendocrine system. It is found in practically all organ systems. Functionally, melatonin-producing cells are part and parcel of the diffuse neuroendocrine system as universal system of a response control and organism protection. Taking into account the large number of such melatonin producing cells in many organs and, the wide spectrum of biological activities of melatonin and specially its main property as an universal regulator of biological rhythm, it is now possible to consider extra pineal melatonin as a key paracrine signal molecule for the local co-ordination of intracellular relationships. Melatonin production from the pineal is regulated by the circadian clock in the suprachiasmatic nuclei and synchronized to 24 hours/day by the daily photo period (Bartness *et al.,* 1993)

Recent research has provided considerable evidence that this evolutionarily conserved signaling bimolecule sub-serves multiple functions in mammals including regulation of circadian rhythms of reproductive responses (Pang *et al.,* 1993; Reiter, 1991). Intriguingly both receptor non-receptor mediated mechanisms have been proposed for the action of melatonin (Reiter, 1996). To date, specific G-proteins (Mel I-A / MT_1, MEL_1B/MT_2, MEL_1C and Mel_2/MT_3) and melatonin receptors (R_2R_2) have been reported (Dubocovich, 1995; Reppert *et al.,* 1996; Wiesenberg *et*

a.l., 1995). In many seasonally breeding species, the timing of initial reproductive development (puberty) is strongly influenced by day lengths experienced during the postnatal period. The influence of melatonin on reproductive development begins during the prenatal development and extends into postnatal life. The primary source of melatonin for the developing mammal is the maternal pineal gland. Maternal melatonin reaches the offspring via milk (Reppert and Klein, 1978). Rhythmic melatonin production from the developing pineal is first significant during the second to third week of postnatal life in rodents (Tamarkin *et al.*, 1980). Administration of melatonin in the appropriate temporal (durational) patterns can influence the timing of puberty in many species. In Syrian hamsters, pinealectomy prevents the seasonal reduction in gonadotropin secretion and gonadal regression normally brought about by experimental or natural short photoperiod (Reiter, 1980). Injection of melatonin reverses the effect of pinealectomy on gonadotropin secretion and causes gonadal regression. Pinealectomy suppresses responses to both long and short photoperiod, and melatonin, depending on its specific pattern, reinstates both these responses. Thus, role of melatonin is to provide an endocrine clock for day length.

Studies have shown effect of melatonin on gonadotropins & other hormones. Melatonin inhibits testosterone secretion by adult rat Leydig cells through reduction of GnRH-induced increase in cytosolic Ca^{+2}

(Valenti, 1999). The effect of exogenous melatonin on circadian rhythm of T_3, T_4, corticosterone and testosterone suggest that exogenous melatonin has the suppressive effect on diurnal secretion of T_3, T_4, and testosterone in pinealectomized rats but stimulates the rhythmical corticosterone secretion (Korczala *et al.,* 1991). Melatonin administration in cyclic old rat females restored the basal exogenous concentrations of pituitary responsiveness to similar values to those observed in young rats. (Diaz *et al.,* 2000). Also the anti-oxidative effect of melatonin inhibits oxidative damage to lipids, caused by the high level of oxidative stress during pregnancy (Sainz, 2000). High levels of oxidative stress induce an increase in oxidative damage to lipids, which in some cases is inhibited by the anti-oxidative actions of pineal indoles (Sainz *et al.,* 2000). During ageing, the effect of melatonin is exerted primarily at the hypothalamic-pituitary axis by restoring the basal concentrations of pituitary hormones and, pituitary responsiveness to values similar to young rats (Diaz, 2000). It is demonstrated that a pineal- directed circadian function and cyclicity is fundamental for the regulation of sexual reproductive physiology and that proper intervention with melatonin may potentially postpone aging of both neural and gonadal sexual function (Pierpaoli *et al.,* 1997). Melatonin provides protection against neuro-degeneration and against mutagenic and carcinogenic actions of hydroxyl radicals (Reiter, 1992, 1993; Hardeland *et al.,* 1993; Poeggeler *et al.,* 1993). One experimental finding

suggests melatonin as a possible tumour marker (Bartsch and Bartsch, 1999). Melatonin is reported to induced sleep and hypo-metabolism (Huim and Zisapal, 1992; Saarela and Reiter, 1994) and also to play a protective role against stress (Miline, 1980). It is also shown that melatonin has suppresent effect on HPA axis activation, decreases body temperature and induces hyperphagia in animals (Raghvendra and Kulkarni, 2000). Thus melatonin possesses anti-oxidant, anti-senescent, anti-pyretic and onchostatic properties (George, 1999). Influence of melatonin on reproduction is well documented in various vertebrate species (Reiter 1981, 1982; Underwood, 1981; Blark *et al.,* 1982, Reiter *et al.,* 1988; Pavet, 1988; Patel and Ramachandran, 1988; Cavallo, 1993; Lewinski, 1993; Weaver, 1993). Non-classical hormones other than melatonin, like insulin (Gizard *et al.,* 1991), growth hormone (Barthett *et al.,* 1990), thyroid hormones (Palmero *et al.,* 1988; Meisami *et al.,* 1992; Hardy *et al.,* 1993; Van Haaster *et al.,* 1993; Cooke *et al.,* 1993, 1994) and corticosteroids (Kalimi *et al.,* 1983) emanating from other endocrine glands also modulate reproductive functions. Of these, the three glands of importance are 1. the adrenal 2. the pineal 3. the thyroid. Stress affects testicular function and steroidogenesis by inducing glucocorticoid increase (Collu *et al.,* 1994; Orr *et al.,* 1994; Masic *et al.,* 1996; Chatterton *et al.,* 1997), more in the developmental stages. The foetal environment is the key determinant of the adult phenotype, being linked

to development of diseases and also the timing of puberty. Glucocorticoid is 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 (Benedicktsson *et al.,* 1993; Levitt *et al.,* 1996; Lindsay *et al.,* 1996), suggesting glucocorticoid involvement in the programming of postnatal systems. Foetal exposure to glucocorticoid may also be detrimental for postnatal reproductive development as shown by the delayed onset of puberty in the female offspring of mothers subjected to stress (Politch and Herrenkohl, 1984) or treated with adreno-corticotrophic hormone (ACTH) during gestation (Harvey and Chevins, 1987) and advanced puberty in male offspring (Smith and Waddell, 2000). Prenatal stress produces long-term changes in the hypothalamus-pituitary-adrenal (HPA) axis in the offspring (Henry *et al.,* 1994). The activity of HPA axis in response to stress in adult offspring is mediated at least in part by a reduction in corticosteroid receptors at specific times of day. Prenatally stressed females exhibits patterns of altered corticosterone secretion and altered temporal functioning of HPA axis, which is not only age specific but also gender specific (Manda *et al.,* 1994; Koehl *et al.,* 1999; King and Edwards, 1999). The increase in glucocorticoid secretion due to stress inhibits gonadal function by decreaseing testicular testosterone production (Collu *et al.,* 1991; Orr *et*

al., 1994; Maric *et al.,* 1996; Chatterton *et al.,* 1997). Men under physiological stress showed an increased cortisol and decreased plasma testosterone associated with a elevation in plasma LH (Chatterton *et al.,* 1997). Lope-Calderon et al, (1990) reported low effect level of LHRH is responsible for the resistant stress induced decrease in gonadotrophin secretion, while, restraint stress for 3 hrs increased corticosterone levels and decreased serum testosterone level without affecting LH (Orr and Mann, 1990; Orr *et al.,* 1994).

The time and age dependent doses of melatonin have shown different effects on reproductive system. Previous work from this laboratory has shown time independent effect of melatonin on both body and testes growth.

The effects of alterations in glucocorticoid and melatonin levels in the postnatal period remain still an unexplored avenue. Hence, it was thought pertinent to study the long-term effects of experimental alterations in glucocorticoids and melatonin status on growth, attainment of maturation and function of the male reproductive system at prepubertal, pubertal and adult stages along with hormonal profiles.

The objectives defined were:

1) To study the impact of neonatal alterations in hormonal milieu, on growth and maturation of testes in both low and high dose of corticosterone, and metyrapone treated animals.

2) To assess the effect of neonatal hormonal manipulation on the growth and maturation of testes in melatonin treated rats,

3) To study the effect of combination of both corticosterone and/or metyrapone with melatonin, on the postnatal growth and maturation of testes and also the hormonal interplay involving the HHG, HHT and HHA axes.

The experimental paradigms included rendering animals hypercorticalic (time and dose specific), hypermelatonemic, hypercorticalic and hypermelatonemic, hypocorticalic and hypocorticalic and hypermelatonemic for these neonates were injected intra-peritoneally *(i.p.)* with corticosterone in the morning or evening, melatonin in the evening, metyrapone in the morning, and combinations of corticosterone and melatonin or metyrapone and melatonin from day 0 to day 21. The efforts were than assessed in the adult.

Corticosterone excess in the present study suggests that neonatal corticosterone excess in physiological range can have favourable influence, especially steroidogenesis in rats and man. Smaller doses of corticosterone not only hasten puberty but also augment spermatogenesis. Hypercorticalism reduces tubular length and sertoli cell number, but increase germ cell number, due to reduced germ cell apoptosis and degenerative loss, an effect, which is accredited to long-term effect of neonatal exposure. The observations of only a few stages

of spermatogenesis in the sections of tubules lead to a speculation that corticosterone might exert a synchronising effect on initiation of spermatogenesis for longer stretches of tubule which as a consequence should theoretically reduce the number of waves per tubule. Corticosterone excess during the neonatal period appears to have a long-term depressing influence on the set point of hypothalamo-hypophyseal-adrenal axis, as marked by the lower levels of corticosterone titre in the adult (60 to 90 days). The higher corticosterone level during the treatment period persisted ad extended upto at least a month after the cessation of treatment; it may be speculated that neonatal exposure to corticosterone decreases the metabolic clearance of the hormone leading to elevated level, an effect, which persist for sometime after exposure to corticosterone. The hypothalamo-hypophyseal-thyroid (HHT) axis was permanently elevated and the hypothalamo-hypophyseal-gonad (HHG) axis set point is lowered. In the higher dose regimen of corticosterone, the noticeable difference from lower dose regimen, is the premature sloughing off of advanced germ cells especially spermatids and spermatozoa, which is more pronounced in the evening schedule. The higher dose of corticosterone shows a time dependent differential effect on the HHT axis with permanently elevated set point with morning schedule and an opposite lowered set point in the evening schedule. The HHG axis set point is also lowered along with the HHA axis in the higher

dose regimen. The decrease in germ cell apoptosis mediated by neonatal elevated corticosterone could be by way of altered secretion of growth/paracrine factors and/or adhesion molecules from Sertoli cells by genetic reprogramming.

The effect of neonatal hypermelatonemia was also investigated. Subsequently, the combination studies were also carried out simultaneously with hyper/hypocorticalism. The body weight of melatonin animals showed increase while the relative testes weight recorded a decrease. Correspondingly, there was decrease in the tubular length, but the germinal epithelial thickness increased. The over all germ population in the adult rat was higher but the degeneration observed was also high. These paradoxical effects observed can be accredited to increase in corticosterone titre leading to decreased germ cell apoptosis and increased degeneration due to melatonin excess. The loss of advanced germ cells observed was because of long-term neonatal melatonin effect on germ cell/Sertoli cell adhesive properties. The HHT axis set point is lowered as in corticosterone treated rats, but the lowered T_4 level 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. Melatonin has a dampening effect on the HHG axis, either directly or indirectly through corticosterone.

The combination treatment of evening hypercorticalism and hypermelatonemia resulted in potentiated deleterious effects of melatonin

and, so apparently favourable influence of corticosterone has potentiating effect on melatonin effect on germ cells. The evening hypocorticalism in combination with hypermelatonemia can either up regulate the GnRH pulse regulator and/or up regulate the sensitivity of pituitary and lowered testosterone level at least to a probable reduced sensitivity of pubertal and post pubertal Leydig cells to LH. The lowered HHA axis could be a direct action of MT or even an indirect one medicated through corticosterone.

The morning hypercorticalism and hypermelatonemia has depicted the same degree of damage. The lowering of HHG and HHA axes set points is probably a consequential effect of the elevated corticosterone level. But the CORT effect on its own axis is not nullified by MT and hence does not show any alteration in MT rats.

As corollary to the hypercorticalism study, neonatal hypocorticalism was studied. It resulted in increased germinal epithelial thickness, total basement membrane area and theoretical germ cell count. The germ cell loss due to degeneration was very high. The serum LH level was high and testosterone level was significantly low. HHT axis showed differential effects as the T_3 and T_4 levels were low but TSH levels were high. This could be due to increase in serum corticosterone level in the prepubertal period. It can be concluded that HHT and HHG axes show increased

sensitivity of pituitary for releasing factors and paradoxically lower sensitivity of the target glands.

In the combination treatment of metyrapone with melatonin, the negative MET influence on germ cell number is nullified. The overall germ cell number is less but the same is significantly increased per meter length of tubule. The HHG and HHA axes set points are lowered but the HHT axis showed differential effect. Whereas the T_4 and T_3 levels were reduced the TSH level was elevated. It can be inferred that the effect of melatonin on growth kinetics of testes constituents is more potentiated in a hypocorticosterone background. Overall, it can be concluded that melatonin-glucocorticoid interaction in neonatal period are of crucial significance in modulating the postnatal growth, attainment of puberty and functional expression of adult testis and accessory organs.