CONCISE SUMMARY

ALTERED NEONATAL MELATONIN AND THYROID HORMONE STATUS ON THE FUNCTIONAL FEATURES OF THE ADULT TESTIS AND OVARY IN THE RAT

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Virtually all organisms that have been studied exhibit circadian rhythms in physiology and behaviour. These rhythms have period lengths close to, but not exactly 24h when the organism is maintained in a time-free environment i.e. an environment that provides no cues for the time-of-day (Pittendrigh, 1993; Goldman, 1999; Hastings *et al.*, 2003). In the process of regulation of these exogenous and endogenous rhythms, "the pineal acts as an intermediary between the environment and endocrine system and melatonin act as a chemical link". This shows that mammalian pineal gland acts as a neurotransducer converting neural signals, specifically ambient lighting conditions and environmental cues, into endocrine message leading to synthesis and release of melatonin from the pineal gland (Stetson and Watson, 1984; Reiter, 1993; Ganguly *et al.*, 2002; Stehle *et al.*, 2002). Melatonin secretion pattern is modulated by the photoperiod and thereby provides both a daily and seasonal endocrine message, and affects the reproductive activity by regulating the activity of hypothalamic neuroendocrine circuitry. The physiological role of melatonin in regulating the seasonal reproduction in mammals has been well established (Karsch *et al.*, 1984; Underwood and Goldman, 1987; Foster *et al.*, 1989, Bartness *et al.*, 1993; Weaver, 2000). Melatonin influences sexual maturity as has been demonstrated by the presence of melatonin receptors in reproductive organs

(Lubozhitzky and Lavie, 1999). In rodents, maternal melatonin crosses placenta and provides day-length information to fetuses which along with photoperiodic information that is obtained after birth, influence juvenile development.

Melatonin, the main hormone of pineal gland, acts on the neuroendocrine-reproductive axis exerting an antigonadotropic effect (Tamarkin *et al.*, 1985; Glass and Dolan, 1988). However, in some experimental conditions, MT has been shown to induce stimulatory hypophyseal-gonadal axis (Amadar *et al.*, 1986). Short photoperiod induced regressed testes size, ceased spermatogenesis and atrophy of accessory reproductive organs have been well documented in adult male rats whereas long photoperiods significantly shows increased testicular weights, diameters of seminiferous tubules and serum testosterone levels (Kus *et al.*, 2003). Siberian Hamsters maintained from birth under long photoperiod shows enlarged and functional testes at 35-40 days of age whereas at short photoperiods testicular development is delayed up to 150 days of age (Gaston and Menaker, 19657; Bernard *et al.*, 1997). Juvenile Siberian Hamsters show increased testes size to that of adults by 35 days of age on long photoperiods and small and underdeveloped testes at short photoperiods (Gunduz and Stetson, 1994). Exposure to bright light, suppressing the concentration of melatonin in circulation, is hypothesized to be

useful in treatment of both male and female infertility in couples with abnormal melatonin metabolism (Partonen, 1999).

Pinealectomy attenuates and reveres the effects of food and sensory deprivation; it can accelerate pubertal maturation in neonatally healthy rats and is also known to increase Prolactin (PRL) secretion in some mammals (Clarke, 1980). Pinealectomy (Px) has been known to prevent the seasonal reduction in gonadotropin secretion and gonadal regression brought about by experimental or natural short photoperiod and also shown to increase reproductive activity in mammals (Reiter, 1973, 1980; Reiter *et al.*, 1983). Exogenous melatonin administration reveres the effect of Px on gonadotropin secretion and causes gonadal regression. Plasma melatonin concentration is found to be almost nil (undetectable) after pinealectomy. Pinealectomy suppresses responses to both long and short photoperiod, and melatonin, depending on its specific pattern, reinstates both these responses. Recently it has been shown that there is increased incidence of spontaneous malformations in pups and increased litter size from pinealectomised dams (Takashi *et al.*, 2004). Pinealectomy suppresses responses to both long and short photoperiod, and melatonin, depending on its specific pattern, reinstates both these responses. The effect of exogenous melatonin on circadian rhythm of T3, T4, corticosterone and testosterone secretion suggest that exogenous melatonin has the

suppressive activity on diurnal secretion of T3, T4 and testosterone in pinealectomized rats but stimulates the rhythmical corticosterone secretion (Korczala *et al.*, 1991). Melatonin is found to have a general inhibitory effect on thyroid hormones (Wright et al., 1996; Lewinski, 2002; Mogulkoc and Baltaci, 2002). It is known that melatonin administration inhibits thyroid growth, it is stimulated by pinealectomy (Px) and that MT supplementation reverses this condition in pinealectomized rats (Wajs and Lewinski, 1992).

It is shown that melatonin can alter the morphology, steroidogenesis or cGMP production of testicular tissues, Leydig cells (Ellis, 1977; Ng and Lo, 1988; Persengiev and Kehajova, 1991; Niedziela *et al.*, 1995; Valenti, *et al.*, 1997) and of corpus luteum and granulosa cells *in vitro* (Mac Phee *et al.*, 1975; Fiske *et al.*, 1984; Baratta and Tamanini, 1992; Marayama *et al.*, 1997). These findings suggest the direct action of melatonin on the testis and ovary. Melatonin is known to have differential influence on male and female reproductive activity. A higher concentration of MT is detected in the human follicular fluids than in serum (Brzezinski *et al.*, 1987). There are varied reports of melatonin action on female reproductive system in rats. Melatonin treatment during gestation in the rat produced delayed sexual maturation and vaginal opening in female offsprings (Colmenero *et al.*, 1991). Notable effects of exogenous melatonin in adult female rats include disruption of

normal estrous cycles and reduced fertility (Hastuta *et al.*, 2004). Pinealectomy leads to morphological alterations of rat ovaries with functional changes in steroidogenesis and a decrease in progesterone receptor expression (Soares *et al.*, 2003). Functional pinealectomy by continuous light in Indian desert gerbil showed regression of all stages of follicular growth and also reduced number of small antral follicles (Sinhasane and Joshi, 1997).

Other than gonadal hormones, thyroid hormones too have definite influence on functions like reproduction, growth, differentiation and metabolism. The two basic biological functions of thyroid hormones (T3 and T4) are (1) effect on cellular differentiation and development, and (2) effect on metabolic pathways that are interconnected. The majority of effects of thyroid hormones are now believed to be mediated through interactions of thyroxine with its nuclear receptors, so as to bring about changes in gene expression (Lagu, 2001). Thyroid hormone enhances effects produced by other hormones and therefore alters nitrogen balance and is either protein anabolic or catabolic.

Thus, co-operative role of thyroid hormones, produce changes in target tissues allow these tissues to be more responsive to another hormone, to neural stimulation or possibly to certain environmental stimuli such as light. Thyroid hormones deficiency (hypothyroidism) may result in a wide variety of clinical and

physiological disturbances in virtually every organ system. Involvement of thyroid axis and reproduction are the two most important endocrine functions that have been often linked through positive or negative thyroid-gonadal interrelationship and in most species studied thyroid activity follows an annual cycle closely related with sexual cycle (Assenmacher and Boissin, 1986). Thyroid hormones also have documented actions on the secretion of hormones involved in reproduction and maintenance of pregnancy. The lack of thyroid hormones has been known to produce reproductive abnormalities including irregular menstrual cycle, amenorrhea and galactorrhea accompanied by increased prolactin secretion in women (Onishi *et al.*, 1977; Honbo *et al.*, 1978; Shahshahani and Wong, 1978).

The role of thyroid hormones in testicular physiology is not yet totally understood but their effects on female reproductive system are well established (Stardtman, 1993; Longscope, 1996). Neonatal transient hypothyroidism induces precocious puberty and macroorchidism associated with an increase in daily sperm production and, in Sertoli, Leydig and germ cell numbers (Anasti *et al.*, 1995; Bruder *et al.*, 1995; Cooke *et al.*, 1991, 1992; Cooke and Meisami, 1991; Hardy *et al.*, 1993, 1996; Hess *et al.*, 1996, Van Haaster *et al.*, 1992; Ramachandran *et al.*, 2004). However, recent findings by Buzzard et al. (2000), demonstrate the thyroid hormone

receptor expression in germ cells undergoing spermatogenic differentiation, which suggests a possible role for thyroid hormone even in the adult rat testis. Also, demonstrations of T_3 receptor mRNA in Leydig cells of immature and mature rats (Hardy *et al.*, 1996) give further idea about thyroid-testis relationship. The impact of hypothyroidism varies depending upon the age of affected subjects, species as well as age of induction, sex, photoperiod, temperature, duration and severity of the disease. This can be correlated with higher concentration of thyroid hormone receptors during pre-pubertal and pubertal period as the number of receptors decreases in testes after puberty.

Recent findings have shown that thyroid hormones have significant functional relationship with testis, in particular with Leydig cell differentiation, proliferation and stimulation of Leydig cell steroidogenic function (Handagam-Mendis and Ariyaratne, 2004), direct effects on the development of prepubertal testes and the regulation of FSH R and ABP gene expression in Sertoli cells, as well as the LHR mRNA levels in Leydig cells; all of which show that thyroid hormones have modulating effects on gonadotropin control of testis functions (Rao *et al.*, 2003).

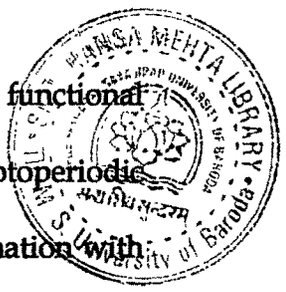
In adult rats, hypothyroidism influences normal follicular maturation of the ovary and gonadotropin secretion, resulting in irregular estrous cycles as a result of hypersecrtion of progesterone

due to increase in prolactin levels which causes prolong periods of diestrous, suppression of LH secretion and follicular maturation while T3 treatment restores normal estrous cycle and ovarian functions (Ortega *et al.*, 1990; Mattheji *et al.*, 1995; Armada-Dias *et al.*, 2001; Tohei, 2004). Hypothyroidism in pre-pubertal rats showed disturbed folliculogenesis, that hampers the differentiation and not the proliferation of granulosa cells (Dijkstra *et al.*, 1996). Thyroxine administration to pre-pubertal rats (day 21-29) showed improved follicular development but does not effect the gonadotrophin secretion.

Stress induced adverse effects on reproduction is known to be primarily mediated through higher concentration of glucocorticoids (GC) (Cooke *et al.*, 2004). Influence of GCs on mammalian reproduction is complex and known to be dose-dependent. Physiological levels of GC are usually beneficial and necessary for normal functioning of the reproductive system at various levels or stages of development. Excess of GC on the pituitary-gonadal axis of males and females as well as the developing fetus may be detrimental (Cooke *et al.*, 2004). GC exerts various deleterious effects on the interstitial Leydig cell of the testis, including direct inhibition of testosterone biosynthesis, suppression of LH receptor expression, testicular germ cell apoptosis and induction of Leydig cell apoptosis (Bambino and Hsueh, 1981; Monder *et al.*, 1994;

Yazawa *et al.*, 2000; Sasagawa *et al.*, 2001; Gao *et al.*, 2002). Recent data also reveals that adreno-cortical-testicular interaction in the rat is influenced by age and stress type (Cooke *et al.*, 2004). Cold stress produced a decrease in serum testosterone, whereas restraint stress increases the serum T level in 60-day old rats (late puberty) and such a stress quality related differences were not observed in 40 day old rats (early puberty) (Gomez and Dallman, 2001; Gomez *et al.*, 2002). Prenatal stress produces long-term changes in the HPA axis in the offspring (Henry *et al.*, 1994). The activity of HPA axis in adult offspring is mediated at least in part by a reduction in corticosteroid receptors at specific time of day. This indicates that prenatal stressed rats exhibit an altered temporal functioning of the HPA axis, which is not only age specific (Henry *et al.*, 1994) but also gender specific (Handa *et al.*, 1994; Koehl *et al.*, 1999; King and Edwards, 1999).

The effect of functional manipulations of thyroid or pineal individually has not been uniform and even more confusing and ill defined are the interactions between these endocrine glands and their role on reproductive functions. Variable effects of pineal on adrenal activity with respect to reproductive functions have been reported in several mammalian species (Motta *et al.*, 1971; Vaughan *et al.*, 1972; Ogle and Kitay, 1976; Nir, 1978; Volrath. 1981; Jimnez *et al.*, 1993).



The disparate and often contradictory reports on the functional ability of these endocrine glands under photoperiodic manipulations and their mechanism of action in combination with each other have provided the necessary impetus for the present work.

Study on neonatal hypermelatonemia by melatonin administration revealed increased number of germ cells but loss of advanced germ cells by sloughing off. Neonatal hypermelatonemia also permanently lowered HHG axis but elevated the HHT axis though T4 levels were reduced in adults (Ramachandran *et al.,* 2004). Neonatal hypo/hyperthyroidism showed differential effects on adult testis functions with increased testes size and germ and Sertoli cell numbers and, decreased adult testes size, Sertoli cell number and sperm mass respectively (Lagu, 2001). Studies on neonatal exposure to glucocorticoids have shown dose and time dependent long-term effects on adult testis function and neuroendocrine axes. Optimum levels of corticosterone excess during neonatal period produced favourable influence on adult spermatogenic functions whereas hypocorticalism showed decreased germ cell number and sperm density in the adult testis (Bhavsar, 2001). Another study from also showed that neonatal pinealectomy results in increased adult testes size and accessory sex organs (Sharma, 1996).

Results from our laboratory have suggested that neonatal hormonal disturbances can bring about possible favourable/unfavourable influences, which bring about permanent long-term consequences on the developmental neuroendocrine axes. There are no studies on alterations in melatonin, thyroid or corticosterone levels in relation to photoperiodic manipulations in the neonatal period on male adult reproductive functions. Hence, it was thought pertinent to study the long-term effects of experimental alterations in melatonin, thyroid and corticosterone status on growth, attainment of maturation and function of the testes at prepubertal, pubertal and adult stages along with hormonal profiles. Also, the long-term consequences of melatonin and thyroid interactions have been extended to female reproductive system to understand whether MT and thyroid hormones have possible favourable/unfavourable influence on adult ovarian structure and function.

On this presumption, studies were undertaken from this laboratory to assess the impact of neonatal manipulation involving thyroid, pineal and adrenal hormones on adult testes functions.

In the present study, light induced functional pinealectomy, by exposure of male pups of Charles Foster strain of rats to continuous light alone and in combination with melatonin, hypothyroidism and hypercorticalism on adult testis histoarchitechture on serum hormone titres was assessed. An attempt was also made to see the

possible impact of neonatal manipulations in females as opposed all the above and past studies on males. Influence of neonatal hypermelatonemia, hypothyroidism and combined effect of hypermelatonemia and hypothyroidism on adult ovarian histoarchitectural dynamics and hormone profiles were assessed. 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. Neonatal functional pinealectomy creating a hypomelatonemia 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 lower TSH level, it is inferable that there is increased sensitivity of thyroid gland to TSH. Another significant observation is reduced number of germ cells and 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.

Simultaneous melatonin treatment to LFPx rats in the neonatal period, a decreased testes weight was recorded. 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 increase 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).

The combined effect of LFPx and hypothyroidism showed reduced body weight and increased adult testes weight A novel observation however was the more than doubled increase in tubular length seen in LFPx+HPOT animals. 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. The up regulation of HHT axis is quite distinct to be a combination effect of light and hypothyroidism.

The present study on LFPx+CORT seems to have more potentiated effect on the apoptotic loss of late/mature class of germ cells alluding to a combination effect. LFPx+CORT also seem 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.

Neonatal MT treatment showed lesser body and ovarian weights. 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. The increased estrogen:progesterone ratio as well as increased T3 and T4 levels even in the prepubertal period are favourable changes for follicular health.

Neonatally hypothyroid rats showed more atretic follicles, decreased estrogen:progesterone ratio and ovary weight. Neonatal hypothyroidism seems to have long-term effects on inducing follicular apoptosis.

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 the increased atresia and reduced number of antral follicles and corpora lutea. 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. Further searching investigations are needed to embellish the present observations on a firmer edifice.

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