INTRODUCTION

Survival and reproduction in organisms involve adjustments to changes in the environmental cues by way of entrainment of endogenous rhythms in tune with the circannual and circadian variations. The exact mechanisms, by which environmental information is integrated, are not yet known, but the possible role of pineal gland in long-term adaptation of seasonal reproduction is described in many animals (Reiter, 1991; Vivien-Roels, 1985; Pevet, 1986). The vertebrate pineal organ, also known as the epiphysis cerebri, was described as ".....perhaps the great mystery in the physiology of mammalian organs" by Wurtman and Axelrod, (1965). However, in the past 30 years, sufficient structural and functional data have been obtained to provide a firm basis from which the functional significance of this epithalamic appendage can be unravelled. Pineal organ is known to be present in all vertebrates, except crocodiles, hagfishes, sloths and anteaters. The vertebrate pineal organ is the principal source of circulating melatonin, a methoxy-indole hormone, which is also called the 'hormone of darkness', as it follows a circadian rhythm with high levels at night (Reiter, 1991, Krause and Dubocovich, 1990) and the duration of which is regulated by photoperiod. The circadian release of melatonin is controlled by the master biological clock within the suprachaismatic nucleus and synchronized to 24 hr/day by the

daily photoperiod (Bartness *et al.,* 1993). A major physiological role of melatonin in adult mammals is the regulation of seasonal reproduction (Cardinali, 1981; Karsch *et al.,* 1984; Underwood and Goldman, 1987; Foster *et al.,* 1989; Bartness *et al.,* 1993; Morgan *et al.,* 1994).

Melatonin ultimately affects reproductive activity by modulating the activity of hypothalamic neuroendocrine circuits, whose activity is necessary for gonadal function. The influence of melatonin on reproductive development begins during the prenatal period and extends into postnatal life. The inhibitory effect of melatonin on the neuroendocrine reproductive axis of adult mammals has been well-documented (Wurtman *et al.,* 1963; Hoffman and Reiter, 1965; Rust and Meyer, 1969; Goldman *et al.,* 1976; Lynch and Epstein, 1976; Turek *et al.,* 1976; Vaughan *et al.,* 1976; Kunderling *et al.,* 1979; Reiter, 1980; Cardinali, 1981). The adult male rat has been shown to be insensitive to melatonin while, immature rat has been shown to be sensitive to melatonin by their decreased reproductive organ weights (Motta *et al.,* 1967; Debeljuk, 1969; Rust and Meyer, 1969; Kinson and Robinson, 1970; Kinson and Peat, 1971; Goldman *et al.,* 1976; Lynch and Epstein, 1976; Turek *et al.,* 1976; Vaughan *et al.,* 1976; Kunderling *et al.,* 1979; Reiter, 1980; Cardinali, 1981). In many seasonal breeding temperate species, the timing of puberty is strongly influenced by the photoperiod experienced during the postnatal phase. Administration of melatonin in the approximately temporal patterns can intervene with the

timing of puberty. In Siberian hamsters, exposure to long days stimulates postnatal reproductive development and, short days suppresses. These photoperiodic requirements for pubertal development are transduced to the neuroendocrine axis by melatonin (Yellon and Longo, 1986; Foster *et al.,* 1989; Wilson and Gordon, 1989; Horton *et al.,* 1990; Shaw and Goldman, 1995).

The length of the nocturnal secretion of melatonin reflects the duration of night and it regulates the pulsatile secretion of gonadotrophin releasing hormone (GnRH) from the hypothalamus, involving a complex network of interacting neurons. Changes in GnRH release induces corresponding changes in leutinising hormone secretion, which is responsible for the alternating presence or absence of ovulation in the female, and varying sperm production in the male. In Syrian hamsters, pinealectomy prevents the seasonal reduction in gonadotropin secretion and, the 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. This led to the concept of the anti-gonadal action of melatonin. In neonatal rats, Martin and Sattler (1979) and Martin *et al.* (1980) demonstrated an acute inhibitory effect of melatonin on pituitary LH and FSH responses to LHRH. Melatonin is also reported to diminish ovarian and uterine weight in immature rats (Wurtman *et al.,* 1963; Motta *et al.,* 1967) and, to retard testicular accessory

sex organ development (Debeljuk, 1969; Kinson and Robinson, 1970; Kinson and Peat, 1971). The normal LH rise prior to ovulation is also inhibited by exogenous melatonin (Longnecker and Gallo, 1971; Reiter and Sorrentino Jr., 1971) .The inhibitory effect of melatonin on sexual maturation is reported to be dose dependent in continuous breeders (laboratory rat), when given daily in the afternoon from 20-40 days of age (Lang *et al.,* 1983). In contrast, melatonin has no significant effect when injected during the prepubertal period from 5 to 20 days or adulthood from 70 to 90 days (Lang *et al.,* 1983). Other workers have shown that the time of administration during the photophase is critical for melatonin action (Reiter *et al.,* 1976; Tamarkin *et al.,* 1976; Margolis and Lynch, 1981; Patel and Ramachandran, 1992). Effect of melatonin injections during scotophase have been reported to be inhibitory to testicular growth in hamster (Tamarkin *et al.,* 1976) and, Margolis and Lynch (1981) observed a gradual anti-gonadal effect with a peak at the end of the photophase, when white footed female mice were administered melatonin at different times during the light-dark cycle (LD 16:8).

The primary source of melatonin for the developing mammal is the maternal pineal gland. The melatonin rhythm in maternal circulation is accurately reflected in the foetus, allowing melatonin to serve as "Chrono-pheromone". Transplacental movement of melatonin is demonstrated in several species including rodents, sheep and non-human primates (Klein, 1972; Yellon and Longo, 1987,1988; Zemdegs *et al.,* 1988; Velazquez *et al.,*

1992; Strother *et al.,* 1998) and maternal pinealectomy abolishes the rhythm of melatonin in fetal circulation (Nowak *et al.,* 1990; Yellon and Longo, 1988). Maternal melatonin after birth, reaches the offspring through milk (Reppert and Klein, 1978) and the maternal rhythm is maintained in the pups (Velazquez *et al.,* 1992). Rhythmic melatonin production from the developing pineal is first significant during the second to the third week of postnatal life in rodents (Tamarkin *et al.,* 1980). In sheep and human, rhythmicity of melatonin levels in serum originating in the developing pineal gland also begins during the postnatal period (Attanasio *et al.,* 1986; Claypool *et al.,* 1989; Nowak *et al.,* 1990)

Melatonin being a key factor in the regulation of seasonal variation in gonadal activity, the circadian disturbance related to reproduction is probably subsequent to the seasonal change. Moreover, melatonin might be considered essential for both spermatogenesis and folliculogenesis. Exposure to bright light, suppressing the concentration of melatonin in circulation, is hypothesised to be useful in treatment of both male and female infertility in couples with abnormal melatonin metabolism (Partonen, 1999). Melatonin is also identified in extra pineal tissues like retina, the harderian gland, gut mucosa, cerebellum, airway epithelium, liver, kidney, adrenals, thymus, thyroid, pancreas, carotid body, placenta, endometrium, ovary (Kvetnoy, 1999; Stefulji *et al.,* 2001) and testis (Tijmes, 1996). It has also been localized in non-neuroendocrine cells such as mast cells, natural killer cells,

eosinophilic leucocytes, platelets and endothelial cells (Kventnoy, 1999) The list of cells indicates that melatonin has a unique position among the hormones of the diffuse neuro-endocrine system. It is found in practically all organ systems. Functionally, melatonin-producing cells are part and parcel of diffuse neuroendocrine system as a universal system of 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 rhythms, it is now possible to consider extra pineal melatonin as a key paracrine signal molecule for the local co-ordination of intercellular relationships. Melatonin has also been reported to possess antioxidant, anti-senescent, and onchostatic properties (George, 1999). 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). Exogenous melatonin in middle age results in stimulatory effect on pituitary responsiveness to LHRH, indicating improved function of the neuroendocrine-reproductive axis (Diaz *et al.,* 1999). The effect of melatonin on circadian rhythms and on visual, cardiovascular, and reproductive function is believed to be mediated primarily through activation of G-protein coupled receptors (MEL_{1A}/MT_{1A}, MEL_{1B}/MT_2, MEL_{1B}/MT_2, MEL_{1C} and ML_2/MT_3) and nuclear melatonin receptor (RZRα) (Dubocovich, 1995; Wiesenberg *et al.,* 1995; Reppert *et al.,* 1996). Melatonin receptors have also been identified in the epididymis of rat, and it is postulated that one of the possible physiological functions of melatonin is stimulation of MT_1 and MT_2 receptors resulting in inhibition of cAMP signalling and an increase in epithelial cell proliferation (Shiu *et al.,* 2000).

Melatonin induces sleep and hypo-metabolism (Haim and Zisapal, 1992; Saarela and Reiter, 1994). One experimental finding suggests melatonin as a possible tumour marker (Bartsch and Bartsch, 1999) and, melatonin also plays protective role against stress (Miline, 1980). It is also shown that melatonin has suppressant effect on HPA axis activation, decreases core body temperature and induces hyperphagia in animals (Raghvendra and Kulkarni, 2000). Stress affects testicular function and steroidogensis by inducing glucocorticoid increase (Collu *et al.,* 1994; Orr *et al.,* 1994; Maric *et al.,* 1996; Chatterton *et al.,* 1997), more so in the development stages. The foetal environment is the key determinant of the

adult phenotype, being linked to development of diseases, including hypertension and non-insulin dependent diabetes (Phillips *et al.,* 1996) as well as the timing of puberty. Such links may be related, in part, to the level of foetal exposure to maternal glucocorticoid *in vitro* (Baxter and Rousseau, 1979; Benedicktsson *et al.,* 1993; Levitt *et al.,* 1996; Lindsay *et al.,* 1996a, 1996b) via direct actions on the hypothalamic-pituitary-gonadal (HHG) axis and possibly by acting at all the 3 levels (Smith and Waddell, 2000). Foetal glucocorticoid exposure may be an important determinant of postnatal reproductive development because, puberty onset is delayed in the offspring of mothers subjected to stress (Politch and Herrenkohl, 1984) or treated with ACTH during pregnancy (Harvey and Chevins, 1987). Increased exposure of foetus to endogenous maternal glucocorticoids delayed puberty in female offspring, whereas an experimental reduction in fetal glucocorticoid exposure advanced puberty in male offspring (Smith and Waddell, 2000). Reduced exposure of foetus to endogenous glucocorticoids (by maternal metyrapone treatment) enhanced birth weight, and this subsequently led to advanced puberty onset in males (Smith and Waddell, 2000). Peripubertal plasma LH was elevated in male offspring of metyrapone-treated mothers, consistent with their advanced puberty, but in puberty-delayed groups plasma LH appeared unaffected (Smith and Waddell, 2000). Prenatal stress produces long-term changes in the hypothalamus-pituitary-adrenal (HPA) axis in the offsprings (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. Results also show that prenatal stress alters the pattern of corticosterone secretion in pregnant females. 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).

Stress inhibits gonadal function in various animal species including human. Stress–induced increase in glucocorticoids, decreases testicular testosterone production (Collu *et al.,* 1991; Orr *et al.,* 1994; Maric *et al.,* 1996; Chatterton *et al.,* 1997). The effect of psychological stress on testosterone secretion was studied in men preparing for skydiving. These men showed an increase in the level of cortisol and decrease in plasma testosterone associated with an elevation in plasma LH (Chatterton *et al.,* 1997). It has been shown that restraint stress for 3 h increased corticosterone and decreased serum testosterone level without affecting serum LH (Orr and Mann, 1990; Orr *et al.,* 1994). However, Lopez-Calderon *et al.* (1990), reported that low level of hypothalamic leutinising hormone releasing hormone (LHRH) is responsible for the restraint stress induced decrease in gonadotrophin secretion. Thus, the indirect adverse effects of glucocorticoids on testicular testosterone production were likely to be mediated through defective hypothalamo-pituitary axis.

Different timed and, age dependent doses of melatonin have been worked out by different workers with different effects. A time specific inhibitory effect of melatonin on sexual maturation of rat has been demonstrated when administered in the afternoon between 20-40 days of age (Lang *et al.,* 1983) but no effect was seen when melatonin was administered between 5 and 20 days of age or 70 and 90 days of age (Lang *et al.,* 1983). Though a delay in sexual maturation was observed due to melatonin treatment between 20 and 40 days of age, these animals were found to be normal as adults in terms of reproductive function (Lang *et al.,* 1983). A previous work in our laboratory has reported time independent influence of melatonin (morning and evening) on both body and testis growth when given between 10 and 25 days of age (Patel and Ramachandran, 1992).

The effect of alterations in glucocorticoid or melatonin levels in the neonatal period remains still an unexplored avenue/area. Hence, it was thought pertinent to study the long-term effects of experimental alterations in glucocorticoid 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.