Photoperiodically modulated melatonin has been established to synchronize seasonal reproductive status by acting at multiple levels of the HPG axis. However, the direct effect of melatonin at testicular level along with the involvement of opioidergic system in photoperiodic regulation of testicular steroidogenesis is not known. Therefore, under the first theme, mechanisms underlying seasonal regulation of testicular androgen biosynthesis were investigated. Being a metabolically active tissue containing high levels of polyunsaturated fatty, testis is susceptible to oxidative damage that increases under metabolic stress, with increasing chronological age and glucocorticoid-induced stress conditions. Therefore, under the second theme we aimed at delineating the cyto-protective attributes of melatonin against testicular oxidative damage and germ cell apoptosis under different experimental condition(s).

The present dissertation explored the direct effect of melatonin in seasonal regulation of testicular function(s) and the efficacy of melatonin in potentiating protection against testicular oxidative stress for the maintenance of male reproductive status in golden hamster, *Mesocricetus auratus*. Further, the expression analyses of MT1R and µOR in testes under different photoperiodic regimes explored the involvement of melatonergic and endorphinergic systems in fine tuning seasonal variation in testicular steroidogenesis. Moreover, the role of melatonin in regulation of seasonal variation in testicular oxidant-antioxidant status was also explored along with the cyto-protective potential of testicular melatonin under metabolic stress, reproductive senescence and clinical condition have been established. The study was divided into following six chapters:

*Chapter-I: Photoperiodic regulation of melatonin membrane receptor (MT1R) expression and steroidogenesis in testis of adult golden hamster, Mesocricetus auratus. (Mukherjee A and Haldar C, J Photochem Photobiol B, 2014, 140, 374-380)*

Photoperiodic modulation of melatonin membrane receptor (MT1R) expression in testis has never been reported for any seasonal breeder. Thus, the aim of the present study was to investigate the expression dynamics of MT1R in testis and its
interaction with testicular steroidogenesis in a long-day breeder, *Mesocricetus auratus*. Hamsters were exposed to different photoperiodic conditions i.e. critical- (CP; 12.5L:11.5D); short-day- (SD; 8L:16D) and long-day- (LD; 16L:8D) for 10 weeks wherein testicular steroidogenesis, local melatonin synthesis and the expression of MT1R were analyzed. SD induced melatonin suppressed testicular steroidogenesis as evident from regressed testicular histoarchitecture, decreased expression of AR, StAR, LH-R, P450\textsubscript{SCC} and enzyme activities of 3β-HSD and 17β-HSD. Differential photoperiodic regulation of MT1R expression in testis suggests its involvement in photoperiodic signal transduction for seasonal adjustment of reproduction. Increased AA-NAT (Arylalkylamine N-acetyl transferase) activity and local testicular melatonin under SD condition suggest an inhibitory effect of the local melatonergic system on testicular steroidogenesis. Completely opposite responses were recorded for all the parameters analyzed when hamsters were exposed to CP or LD conditions. In conclusion, we may suggest that photoperiod via regulating circulatory and local melatonin level as well as MT1R expression in testes fine tunes the steroidogenesis and thereby, the reproductive status of male golden hamster.

**Chapter-2: Effect of naltrexone in photoperiodic regulation of testicular steroidogenesis in adult golden hamster, *Mesocricetus auratus* (Mukherjee A and Haldar C, Gen Comp Endocrinol; Communicated)**

Photoperiod regulates testicular steroidogenesis through modulation of MT1R expression and local melatonin content. However, deeper insights may unravel additional mediators besides the local melatonergic system in photoperiodic control of testicular steroidogenesis in golden hamster. Endogenous opioid peptides (EOP) comprise a family of neurotransmitters profoundly affecting reproduction through acting at multiple levels of the hypothalamo-Pituitary-Gonadal (HPG) axis. The presence of β-endorphin, a naturally occurring opioid peptide, and its receptor (μ-opioid receptor, μOR) has been reported in testes; however, the functional significance of photoperiodic regulation μOR in testicular steroidogenesis is not clear. In the present study, we investigated the effect of Naltrexone (Nal), a μOR antagonist, in photoperiodic regulation of testicular steroidogenesis. Immunohistochemical studies suggest strong
immunoreactivity for μOR under SD (Short-Day) condition, whereas weak immunoreactivity was observed under LD (Long-Day). The expression of μOR was significantly decreased following Nal administration in all the photoperiodic conditions. Nal administration significantly increased the expression of steroidogenic markers under SD condition as compared to SD-control. Increased enzyme activities for 3β-HSD, 17β-HSD and plasma testosterone concentration suggest increased testicular steroidogenesis following Nal administration under SD condition. The localization and photoperiod dependent variation of μOR in Leydig cells suggests its involvement in steroidogenesis. In conclusion, the increased steroidogenesis following Nal administration indicates the participation of μOR in mediating the inhibitory effect of melatonin on testicular steroidogenesis.

Chapter-3: Photoperiodic modulation of local melatonin content and oxidative stress in testes of adult golden hamster, *Mesocricetus auratus*.

Photoperiodic regulation of testicular antioxidant status has never been reported for any seasonally breeding mammal. Thus, we investigated the photoperiodic regulation of testicular melatonin content and its correlation with the testicular antioxidant defence system in a long-day breeder, *M. auratus*. Hamsters were exposed to different photoperiodic conditions i.e. critical- (CP; 12.5L:11.5D); short-day- (SD; 8L:16D) and long-day- (LD; 16L:8D) for 10 weeks, wherein the activity of testicular antioxidant enzymes, germ cell proliferation and testicular melatonin content was evaluated. We observed a significant increase in the activity of testicular antioxidant enzymes with a simultaneous decrease in lipid peroxidation under SD condition as compared to LD. Concomitant decrease in the expression of AR and PCNA in testes following SD exposure indicate declined germ cell proliferation. Further, the differential expression pattern of testicular glucocorticoid receptor (GR) and altered corticosterone level suggest photoperiod-dependent alteration in testicular stress response. The increased AA-NAT enzyme activity and testicular melatonin content under SD condition are in agreement with the augmented testicular antioxidant status and decreased germ cell proliferation thereby establishing the
Summary

anti-oxidative and anti-gonadotropic attributes of melatonin. Completely opposite responses were recorded for all the parameters analyzed when hamsters were exposed to CP or LD conditions. In conclusion, we may suggest that elevated levels of testicular melatonin and MT1R expression in testes might contribute to the elevated testicular antioxidant status under SD condition. However, elevated level of testosterone under LD condition maintains testicular oxidative stress below the physiological toxic threshold. Therefore, a trade-off relationship between testosterone, melatonin and their receptor might be responsible for the fine tuned regulation of seasonal oxidant-antioxidant profile in testes.

Chapter-4: Role of photoperiod in modulation of 2-deoxy-D-glucose (2-DG) induced metabolic stress on testicular steroidogenesis and antioxidant status in golden hamster, Mesocricetus auratus (Mukherjee A and Haldar C, J Photochem Photobiol B, Under review)

Individuals of many species experience marked seasonal variation in environmental conditions and must adapt to potentially large fluctuations in energy availability and expenditure. Seasonal variation in reproduction has evolved as an adaptive mechanism to minimize maternal cost-to-benefit ratio for better survival of offspring. It is well established that photoperiod regulates testicular steroidogenesis through modulation of circulatory and local level of melatonin and MT1R expression in hamster testes. However, the impact of energy availability in regulation of testicular steroidogenesis and antioxidant status has never been investigated for any seasonal breeder in general and especially for golden hamster. Therefore, the goal of the present study was to assess the role of energetic trade-offs associated with seasonal variation in testicular steroidogenesis and oxidant-antioxidant profile. In this study we experimentally reduced energy availability via administration of the metabolic inhibitor 2-deoxy-D-glucose (2-DG), a synthetic glucose analogue, in critical-, long- and short-day housed golden hamsters (Mesocricetus auratus) and then examined testicular steroidogenesis and oxidant-antioxidant status. 2-DG decreased testicular steroidogenesis and antioxidant enzyme activity in CP and LD exposed hamsters as compared to CP- and LD-control groups. Moreover, a decrease in the plasma- and local- melatonin
level and MT1R expression in testes with a simultaneous increase in plasma level of corticosterone was noted following 2-DG administration in CP and LD exposed hamsters. In contrast, no significant effect of 2-DG treatment was observed on the parameters investigated under SD condition. Therefore, it can be suggested that reductions in energy availability suppressed testicular steroidogenesis and increased oxidative damage in testis under CP and LD condition whereas SD induced melatonin buffers the glcodeprivation-induced suppression of testicular steroidogenesis and maintain testicular antioxidant status.


Age-dependent decline in the level of melatonin induces nitro-oxidative stress and thereby compromises physiological homeostasis including reproduction. Melatonin acts as a direct free radical scavenger and indirect stimulator of antioxidant enzymes which in turn acts as an anti-aging molecule. However, less information exist regarding the age-dependent variation in local melatonin concentration and MT1R expression in testis and its interaction with testicular steroidogenesis and nitro-oxidative stress in a seasonally breeding rodent *Mesocricetus auratus*. Therefore, we evaluated local melatonin level along with MT1R expression and its possible interaction with steroidogenesis and nitro-oxidative stress in testis of young (6 weeks), adult (15 weeks) and old (2 years) aged hamsters. A significant decrease in local melatonin concentration as well as MT1R expression was observed in testes of hamsters with increased age. Increased expression for StAR, P450<sub>scC</sub>, LH-R and AR with increased testosterone concentration suggests increased testicular steroidogenesis in adult hamsters while it declined in old hamsters. An age-dependent elevation in total nitrite-nitrate ion concentration (NO<sub>X</sub>), iNOS expression and lipid peroxidation (TBARS) in testes with a concomitant decrease in testicular antioxidant enzyme activities (SOD, CAT, GSH-P<sub>X</sub>) was noted. Elevated plasma level of corticosterone with increased GR expression in testes suggests altered stress response with advancing age. Therefore, age-associated reproductive senescence
might be a consequence of declined local melatonin concentration with MT1R expression inducing nitro-oxidative stress and thereby results in diminished testicular steroidogenesis. In conclusion, our results suggest an important role of local melatonin and MT1R expression in regulating testicular aging in golden hamster.


The protective effect of melatonin on Dexamethasone (Dex), an extensively used anti-inflammatory and immunosuppressive synthetic glucocorticoid, induced testicular oxidative stress and germ cell apoptosis was investigated in golden hamster. Hamsters were randomly divided into four groups (n=7): group I – control; group II – melatonin treated (10 mg kg$^{-1}$ day$^{-1}$); group III – Dex treated (7 mg kg$^{-1}$ day$^{-1}$) and group IV – combination of Dex and melatonin. All the injections were administered intra-peritoneally for seven consecutive days. The histopathological changes, specific biochemical markers, including antioxidative enzymes, plasma melatonin level and the markers for germ cell apoptosis were evaluated. Dex administration decreased antioxidant enzyme activities (SOD, CAT, GSH-PX), plasma melatonin level and melatonin receptor (MT1) expression with a concomitant increase in lipid peroxidation (TBARS) and altered testicular histopathology which might culminate into increased germ cell apoptosis as evident from increased Bax/Bcl-2 ratio and Caspase-3 expression. However, melatonin pre-treatment enhanced enzyme activities for SOD, CAT, GSH-PX with a simultaneous decrease in Bax/Bcl-2 ratio and Caspase-3 expression. Our findings clearly suggest that melatonin improved defense against Dex induced testicular oxidative stress and prevented germ cell apoptosis, suggesting a novel combinatorial therapeutic approach for management of male reproductive health.
Fig. 13: Hypothetical diagram summarizing the effect of melatonin and MT1R in regulation of seasonal variation in testicular steroidogenesis along with the cyto-protective attributes of melatonin in testes under different experimental condition(s).