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RESEARCH ARTICLE

Localization and expression of androgen receptor (AR) and melatonin membrane receptor (MT1R) in accessory sex organs of adult male golden hamster, *Mesocricetus auratus*: Modulation by photoperiod.

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Abstract

Photoperiodic variation in localization and expression patterns of androgen (AR) and melatonin receptor (MT1R) on accessory sex organs (ASOs) are still unexplored for any seasonal breeder. In the present study, we assessed the AR and MT1R expression dynamics in ASOs of male golden hamster, *Mesocricetus auratus* exposed to different photoperiodic conditions i.e. critical- (CP; 12.5L : 11.5D); short-day- (SD; 8L : 16D) and long-day- (LD; 16L : 8D) photoperiod for 10 weeks. Under SD condition ASOs regressed and reduced drastically their structural integrity as evidenced from the gross-morphology, weight and histoarchitecture. Differential immunoreactivity for MT1R and AR in ASOs of *M. auratus* suggests a possible involvement of both the receptors in transducing the photoperiodic signals for modulation of ASOs function. Biochemical analyses of functional markers suggest the functionality of ASOs being suppressed when exposed to SD condition that might be due to a concomitant decrease in plasma testosterone level along with AR expression and an increase in plasma melatonin level and MT1R expression. An opposite response was observed in hamsters exposed to LD condition. It is evident that, experimental photoperiod regulated the functional integrity of ASOs as it does in nature via modulation of plasma level of melatonin and testosterone and expression patterns of their receptors. It also suggests the potency of photostimulation on ASOs besides its effect on testes. Thus, photoperiodic induction of ASOs function(s) may be used for improvement of quality and quantity of sperm of some endangered seasonally breeding mammals even during reproductive inactive phase.

Introduction

Melatonin, the principal secretory product of the pineal gland, has been unequivocally established to regulate seasonal reproduction (Malpaux *et al*., 1999) in phylogenetically distant organisms in pro- (Chemineau *et al*., 1992) or anti- (Frungieri *et al*., 2005) gonadotropic manner. In seasonally breeding mammals, the pineal gland transduces photoperiodic signal (Hastings *et al*., 1985) through a rhythmic secretion of melatonin (Reiter, 1980; Goldman and Darrow, 1983) thereby synchronizing seasonal and circadian body physiology (Reiter, 1975; Arendt, 1998). Till date two high-affinity G-protein coupled melatonin membrane receptors (MT1R / MT2) have been cloned and characterized in mammals (Reppert *et al*., 1994; Dubocovich and Markowska, 2005). These are ubiquitously located throughout the central nervous system (hypothalamus and pituitary; Wu *et al*., 2006; Gupta *et al*., 2013), peripheral reproductive organs (Izzo *et al*., 2010) and lymphoid tissues (Ahmad and Haldar, 2010) to regulate seasonal variation in reproduction and immunity, two mega-physiological events in seasonally breeding mammals. Although, the presence of both the receptor subtypes have been reported in testes (Izzo *et al*., 2010), the major reproductive

effects of melatonin is mediated via the MT1R melatonin receptor (Prendergast, 2010). MT2 receptor subtype is naturally non-functional due to the presence of two nonsense mutations within the coding region, suggesting that MT2 is not necessary for the photoperiodic response in Siberian and Syrian hamsters (Weaver *et al*., 1996; Jin *et al*., 2003). Furthermore, Yasuo *et al* (2009) also suggested that MT2 may weaken the photoperiodic responses mediated via MT1R. Therefore, in the present study, we recorded melatonin membrane receptor MT1Rlocalization and expression in ASOs of male golden hamster.

The golden hamster (*Mesocricetus auratus*) is a nocturnal long-day breeder. It is an excellent animal model to study the photoperiodic manipulation of circulatory melatonin levels and thus its role in seasonal reproduction (Steger *et al*., 1985). The annual reproductive cycle of golden hamster has been divided into the inhibitory, sexually quiescent, restoration and sexually active phase (Reiter, 1975) and it has been well established that the sexual cycle of golden hamsters is photoperiod dependent (Hoffman and Reiter, 1965). However, under laboratory conditions it remains sexually active throughout the year as long as they are exposed to ≥ 12.5 h light (Steger *et al*., 1985). The epididymis, seminal vesicles and prostate gland are regarded as the major ASOs and play essential roles in the maintenance of male fertility (Pang *et al*., 1979; Elzanaty *et al*., 2002). The epididymis provides the spermatozoa with motility and fertilizing ability (Setchell *et al*., 1994) whereas, secretions from seminal vesicle and prostate are important to safeguard the spermatozoa and facilitate semen coagulation (Kumar and Majumdar, 1995; Gonzales., 2001).

To the best of our knowledge, very few reports are available explaining the role of photoperiod in regulation of structural and functional dynamics of ASOs in mammals **(**Tahka *et al*., 1997; Gottreich *et al*., 2000**)**. Till now, no report is available suggesting the photoperiodic variation in localization and expression pattern of AR and MT1R in ASOs of a nocturnal long-day breeder, in spite of reports providing considerable evidence for the existence of putative melatonin receptors in the epididymis and prostate **(**Yu *et al*., 1994; Gilad *et al*., 1998**)**. Therefore, in the present study our aim was to delineate photoperiodic modulation of ASOs function(s) and localization / expressional pattern of AR and MT1R in ASOs of male golden hamster, *M. auratus.*

Materials and methods

Ethical statement

All the experiments were conducted in accordance with Institutional Practice and within the framework of experimental animals (Scientific Procedure) Act 2007, of the Committee for the Purpose of Supervision and Control on Experiments on Animals (CPSCEA), Government of India, on animal welfare.

Animal procurement and maintenance

Golden hamsters were procured from Central Drug Research Institute (CDRI), Lucknow, India and colony was developed and maintained in the departmental animal house facility. Hamsters were kept under constant temperature (25 ± 2 °C) and light / dark cycle (Critical photoperiod; 12.5 h light, 11.5 h dark; i.e. lights on 07:00–19:30 h). The animals were maintained in polypropylene cages of equal sizes and provided with commercial rodent pellet and tap-water *ad libitum*.

Photoperiodic exposure

Adult male golden hamsters (average weight: 125 ± g; Age: 90-100 days old) were randomly selected and divided into three experimental groups, each containing seven animals (n = 7/group). Each of the experimental groups was exposed to different photoperiodic conditions as follows:

Group I: Critical Photoperiod (CP; 12.5L : 11.5D; light : dark) and considered as control; Group II: Short Day photoperiod (SD; 8L : 16D; light : dark) and Group III: Long Day photoperiod (LD; 16L : 8D; light : dark). They were maintained in the above conditions for 10 weeks. No alterations in the general health status of the animals were noticed throughout the experimental period.

Tissue collection and processing

At the end of the photoperiodic treatment, hamsters were weighed and sacrificed at 22.00 h under deep ether anaesthesia. Trunk blood was collected in a heparinised tube. Serum was separated and stored at - 20 °C. Cauda epididymis, seminal vesicle and ventral prostate were immediately removed, blotted dry and weighed. Tissues were then either quickly fixed in 10 % neutral formalin for histological and immunohistochemical analyses or kept at - 20 °C for biochemical estimations and Western blot analyses.

Histology

After fixation in 10 % neutral buffered formalin for 48 h, tissues were washed under running tap-water to remove extra fixative and subsequently dehydrated. After clearing in xylene, the tissues were embedded in paraffin wax and cut into 6 µm sections. The sections were stretched on clean glass slides pre-coated with 1 % gelatine.

Deparaffinized sections were stained using hematoxylin and eosin (H&E) stain. Histoarchitecture of the *Cauda epididymis*, seminal vesicle and ventral prostate were observed under research microscope (Nikon E 200, Japan) in randomly selected sections.

Immunohistochemical localization of AR and MT1R in ASOs

For immunohistochemical localization, sections were placed on glass slides and were sequentially rehydrated. Then, endogenous peroxide activity was blocked by 0.1 % H_2O_2 in methanol for 30 minutes at room temperature (RT). Sections were washed three times with phosphate buffered saline (PBS; 0.1 M NaH_2PO_4, Na_2HPO_4, NaCl; pH 7.4) and pre-incubated with horse blocking serum (1:100 in PBS; PK-6200, Vector laboratories, Burlinghame, CA) for 2 hours. Then sections were incubated with primary antibodies against MT1R (Mel1aR, R-18, Santa Cruz Biotech, USA, dilution 1: 100) and AR (AR, N-20, Santa Cruz Biotech, USA, dilution 1: 100) for overnight at 4 ^{0}C. Sections were then washed thrice with PBS and incubated with biotinylated secondary antibody (Vectastain ABC Universal kit; PK- 6200, Vector laboratories, Burlinghame, CA, dilution 1: 200). The sections were then washed with PBS and a pre-formed ABC reagent was conjugated to the free biotin of the secondary antibody. The antigens were visualized using the 0.03 % peroxidase substrate 3, 3-diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, USA) in 0.01M Tris-Cl (pH = 7.6) and 0.1 % H_2O_2 and counterstained with Ehrlich's hematoxylin for 5 minutes. Counterstaining with hematoxylin was avoided in case of nuclear antigen i.e. AR. Sections were dehydrated, mounted in DPX and finally observed and photographed under research microscope (Nikon E 200, Japan).

Western blot analysis for AR and MT1R in ASOs

Western blot analysis was performed to check the expression pattern of AR and MT1R in ASOs. Briefly, the testes were homogenized and lysed in RIPA buffer containing aprotinin, sodium orthovanadate and phenyl methylsulphonyl fluoride (PMSF) protease inhibitor cocktail. The protein content of the lysates was estimated. The aliquots containing 100 µg protein were resolved on a 12 % SDS - polyacrylamide gel (PAGE) for melatonin membrane receptor, MT1R and 10 % SDS - PAGE for AR followed by electrotransfer (Biometra, Germany) on nitrocellulose membranes (Bioscience, Keene, NH, USA) for 1 h. Nitrocellulose membranes were blocked for 60 minutes in Tris-buffered saline (TBS; Tris-HCl 50 mM, pH 7.5, NaCl 150 mM) containing 5 % fat free dry milk and were incubated with primary antibodies against MT1R (Mel1aR, R - 18, Santa Cruz Biotech, USA, dilution 1: 200) and AR (AR, N - 20, Santa Cruz Biotech, USA, dilution 1: 200). After overnight incubation at 4 ℃ the membranes were washed thrice with TBS. Immunodetection was carried out using horseradish peroxidase (HRP) conjugated secondary antibody (Donkey anti - goat IgG for MT1R, dilution 1: 500; Goat anti - rabbit IgG for AR, dilution 1: 1,000). Finally, the blots were washed thrice with TBS and developed with Super Signal West Pico Chemiluminescent substrate (34080, Thermo Scientific, USA). Membranes were then stripped with stripping buffer (10 % sodium azide) and β-actin (1: 5,000; A - 2228; Sigma –Aldrich, USA) was used as internal loading control. Densitometric analyses of bands were performed using Image-J analysis software with respect to β - actin.

ELISA for plasma melatonin

The assay was performed according to the manufacturer's protocol provided with the kit (Uscn Life Science Inc. Wuhan, Hubei, USA). The sensitivity of the kit was 4.68 pg/ml —as per the manufacturer. All samples were quantified in a single assay with intra- and inter-assays at < 10 and < 12 % precisions respectively with recovery percentage between 90 and 115.

ELISA for plasma testosterone

The level of testosterone was measured in serum by using a highly sensitive and specific commercial ELISA kit (Dia.Metra, Italy-DK0002) using protocol supplied with kit. The sensitivity of the kit was 5 pg/ml — as per the manufacturer. All samples were quantified in a single assay with intra- and inter-assays precisions were checked and co-efficients of variation were found to be 5.1 % and 7.5 %, respectively.

Determination of alkaline phosphatase activity in the *cauda epididymis*

Activity of alkaline phosphatase activity was estimated following Bessey *et al*. (1946) using p-nitrophenyl phosphate (pNPP) as substrate. Briefly, 0.8 ml of sodium carbonate-bicarbonate buffer (0.1 mol/lit, pH = 10) was added to 2 ml of substrate solution (5 mM/lit) and mixed thoroughly. Then 0.2 ml of epididymal homogenate was added to the reaction mixture. The conc, of the reaction product was measured at 405 nm in a UV-Vis Spectrophotometer against analysis blank.

Determination of fructose concentration in seminal vesicles

Fructose concentration in seminal vesicle was determined following Lindner and Mann, (1960) with minor modifications. Briefly, seminal vesicle was homogenized in 80 % ethanol & centrifuged at 5000 rpm for 10 minutes. To the supernatant, 0.5 ml each of 0.3(N) barium hydroxide and 5 % zinc sulphate were added and were kept in refrigerator for 2 hours. The tubes were then centrifuged again at 5,000 rpm for 10 minutes and the samples were drawn from the supernatant in duplicate. To the samples 2 ml of 0.1 % ethanolic resorcinol followed by 6 ml of 30 % HCl was added and mixed thoroughly. After which the samples were heated to 90 ^{0}C in a water bath for 10

minutes and cooled to room temperature in an ice bath. The optical density was measured within 30 minutes at 410 nm in a UV - Vis spectrophotometer. The amount of fructose present in the sample was determined from a standard curve (10 - 50 µg) with chemically pure fructose.

Determination of sialic acid levels in ventral prostate

The concentration of sialic acid in the ventral prostate was determined according to thiobarbituric acid method (Aminoff, 1961). Briefly, the ventral prostate was homogenised in ice cold 0.1 (N) sulphuric acid (1.0 ml / 10.0 mg tissue weight) and placed in water bath at 80 ^{o}C for 1 hour. The tubes were kept in the refrigerator for 2 hours to allow the tissue fragments to sediment. Thereafter, 0.5 ml of the solution was oxidized with 0.25 ml of periodate reagent for 30 minutes at 37 ^{0}C. Excess of periodate was reduced by adding 0.2 ml of sodium arsenite. As soon as the yellow colour of the liberated iodine had disappeared, 2 ml of thiobarbituric acid reagent (pH = 9.0) was added and the tubes were tightly plugged and kept in boiling water bath for 10 minutes. The solutions were then cooled in ice cold water and 5 ml of acid butanol (butanol containing 5 % v/v 12 N HCl) was added. The solutions were shaken vigorously & the readings were taken from upper separated butanol layer in a UV - Vis spectrophotometer against a blank of glass distilled water. Optical density (O.D.) was recorded at 549 nm and 532 nm, respectively within half an hour of the mixing of acid butanol. Concentration of sialic acid was calculated using Warren's equation = $0.090 \times O.D._{549} - 0.033 \times O.D._{532}$

Statistical analyses

Statistical analysis of the data was performed using SPSS 17.0 (SPSS, IBM, Chicago, IL, USA) with one - way ANOVA followed by Tukey multiple range tests for multiple comparisons. The differences were considered statistically significant when $p < 0.05$.

Results

Weight analyses

Morphometric and weight analyses are among the initial observations to check the effect of any treatment on the target tissue. Weight of the ASOs was recorded to get an overview of the effect of photoperiod on ASOs. Exposure to SD photoperiod caused regression in the gross appearance (Fig. 1) with simultaneous decrease in the weight of epididymis, seminal vesicle and ventral prostate, while LD exposure allowed full morphological development of the ASO. Exposure to SD photoperiod for 10 weeks caused a significant ($p < 0.01$) decrease in weight of ASOs as compared to CP and LD exposed animals, whereas exposure to LD caused significant ($p < 0.01$) increase in ASO weight when compared to CP hamsters (Table 1).

Histological observations

Histological studies indicate that SD condition dramatically suppressed the development of ASOs in male hamster. Whereas CP and LD exposed hamsters showed normal histoarchitecture of ASOs. Histologically, epididymis showed thin epithelial lining with distinct nucleus located basally and lumen filled with sperms in hamsters exposed to CP and LD conditions (Fig. 2A). Lumen of the *cauda epididymis* became totally devoid of spermatozoa in hamsters exposed to SD photoperiod (Fig. 2B). The seminal vesicle (Fig. 2C) and prostate (Fig. 2E) showed normal histoarchitecture with mucosal lining, epithelium with finger like projections pointed towards the lumen which is distended with secretion under CP and LD condition. SD exposure caused excessive inward ramification of the epithelium and lumen contained scanty secretory material of seminal vesicle (Fig. 2D) and prostate (Fig.2F). No significant alterations were noted in the histoarchitecture of the ASOs when comparison was made between LD and CP exposed hamsters and therefore histoarchitecture of the CP group has been excluded.

Immunohistochemical studies

To localize AR and MT1R melatonin receptors in ASOs of hamsters exposed to LD and SD photoperiodic conditions immunohistochemical studies were performed.

Androgen receptor (AR)

Strong nuclear immunoreactivity for AR was observed in both epithelial and stromal cells of the epididymis (Fig. 3A), seminal vesicle (Fig. 3C) and prostate (Fig. 3E) of hamsters exposed to CP and LD photoperiod. The nuclei of the basal as well as luminal epithelial cells displayed a robust deposition of immune reaction products. The secretion in the lumen of seminal vesicle and prostate and spermatozoa in the lumen of the cauda epididymis showed immune reaction non-specifically, as revealed when compared with negative control. Weak immune reaction for AR was observed in both epithelial and stromal cells of the epididymis (Fig. 3B), seminal vesicle (Fig. 3D) and prostate (Fig. 3F) of hamsters exposed to CP and LD photoperiod.

Melatonin receptor (MT1R)

Weak immunoreactivity for MT1R was observed on the membrane of epithelial and stromal cells of epididymis (Fig. 4A), seminal vesicle (Fig. 4C) and prostate (Fig. 4E) in hamsters exposed to CP and LD photoperiod, whereas strong immunoreactivity for MT1R was noted on the membrane of epithelial and stromal cells of epididymis (Fig. 4B), seminal vesicle (Fig. 4D) and prostate (Fig. 4F) of hamsters exposed to SD conditions.

Western blot analyses

To check the expression of melatonin receptor subtype, MT1R, and AR in ASOs i.e. cauda epididymis, seminal vesicle and ventral prostate in *M. auratus* at translational level, western blot analyses were performed. MT1R and AR proteins were detected as single bands between 35-40 KDa and 100-110 KDa respectively, corresponding to the reported molecular mass of the receptor proteins. AR expression in ASOs showed a significant ($p < 0.01$) decrease in SD experienced hamsters when compared with the LD and CP group. Moreover, LD experienced animals showed a significantly ($p< 0.01$) increased AR expression when compared to CP exposed animals (Fig. 5). A significant ($p < 0.01$) increase in MT1R expression was observed in ASOs of SD exposed hamsters as compared to LD and CP animals. Whereas, LD experienced animals showed significant ($p<0.01$) decrease in MT1R when compared to CP exposed animals (Fig. 6).

Hormonal analyses

The plasma melatonin levels were found to be elevated significantly ($p < 0.01$) in SD exposed hamsters as compared to LD and CP groups. On the other hand significantly ($p < 0.01$) decreased peripheral testosterone level was recorded in SD hamsters when compared with LD and CP exposed hamsters. However, LD exposed animals showed significant ($p < 0.01$) decrease in melatonin levels as well as significant ($p < 0.01$) increase in plasma testosterone levels when compared to CP exposed animals (Fig. 7).

Functional status of ASOs

Assessment of functional status of ASOs was performed by determination of alkaline phosphatase activity, fructose and sialic acid concentrations in *cauda epididymis*, seminal vesicle and ventral prostate of hamsters. A significant ($p < 0.01$) decrease in alkaline phosphatase activity, fructose and sialic acid concentrations were observed in SD exposed hamsters. Whereas LD experienced animals showed significant ($p < 0.01$) increase in all the aforesaid parameters when compared to CP exposed animals (Table 2).

Discussion

In the present study, photoperiodic modulation of functional integrity of ASOs were studied in detail along with localization and expressional pattern of AR and MT1R in a nocturnal seasonal breeder, *M. auratus* for the first time. Dysfunction of ASOs in mammals compromises fertility status in males **(**Pang *et al*., 1979; Chow *et al*., 1986**)** and hampers in females the normal early embryonic development of the foetus (W.S.O *et al*., 1988). Therefore, the detailed understanding of regulatory mechanisms involved in the photoperiodic control of ASOs function(s) in male would be of great significance.

Results of the present study indicate that being androgen dependent organs, the epididymis, seminal vesicle and prostate gland showed a parallel response that of testis and plasma level of testosterone under different photoperiodic conditions i.e. inhibitory effects of SD and stimulatory effects of LD photoperiod was noted. Significantly decreased epididymis, seminal vesicle and prostate weight under SD conditions corroborate the earlier reports (Takha *et al*., 1997; Gottreich *et al*., 2000) which were due to the decreased plasma level of testosterone as a prime cause of histological alterations and decreased mass of ASOs. Such responses have also been reported from experimental orchidectomy or treatment with GnRH antagonist or Ethane Dimethane Sulphonate (EDS) (Yang *et al*., 2006). We observed a significantly ($p<0.01$) elevated plasma testosterone level in hamsters exposed to LD and significantly ($p<0.01$) low level in SD exposed ones having a reciprocal relationship with plasma melatonin level supporting the anti-gonadotropic and anti-androgenic actions of melatonin under LD in a seasonal breeder, *M. auratus* that were reported earlier as well (Wu *et al*., 2001; Frungieri *et al*., 2005).

The presence of melatonin receptor, MT1R has been reported in the testis, epididymis and prostate of many rodents as well as for humans (Yu *et al*., 1994; Gilad *et al*., 1998; Ahmad *et al*., 2012; Ahmad and Haldar, 2010b). However, photoperiodic regulation of MT1R expression in ASOs has never been investigated in any seasonal breeder in general and specially in nocturnal long-day breeder, *M. auratus*. We further assessed the localization and relative expression of melatonin receptor, MT1R and AR in the ASOs of hamsters under experimental LD condition which corresponded to the reproductively active and experimental SD condition which corresponded to the reproductive inactive phase. Immunoreactivity for AR and MT1R were localized in the epithelial cells, stromal and smooth muscles of ASOs. Strong immunoreactivity for MT1R, observed under SD condition, corresponding to weak nuclear immunoreactivity for AR in the ASOs thus immunoreactivity for MT1R and AR localization showed an opposite expression pattern during LD condition.

Interestingly, we observed a reproductive phase dependent variation in expression pattern of melatonin receptor, MT1R and AR that might be the key regulators for the photoperiodic induction of reproduction in the male golden hamster. Increased melatonin receptor expression coincided with the elevated plasma levels of melatonin in hamsters exposed to SD regime gets strong support from our earlier reports on a diurnal seasonal breeder (Ahmad and Haldar, 2010a). On the other hand, our results of increased expression of AR during reproductively active phase corresponded to high plasma testosterone level and *vice versa* gets support from the previous studies by Bittman *et al* (2003). The circulatory level of androgen is essential for the stability and maintenance of AR in target tissues (Syms *et al*., 1985; Zhou *et al*., 1995) thus under SD conditions low plasma testosterone was unable to stabilize AR and thus a significantly decreased AR expression was observed and this also explains the reverse scenario observed for the LD exposure.

Biochemical parameters viz. alkaline phosphatase activity, fructose and sialic acid concentration in cauda epididymis, seminal vesicle and prostate showed decrease under SD and increase under LD condition as compared to CP exposed hamsters suggesting a definite stimulatory role of photoperiod in regulation of secretory function of ASO. Alkaline phosphatases are membrane-bound ectoenzymes, possessing variable amounts of sialic acid and play a crucial role in the transport of essential biological molecules between the epithelial principal cells and sub-epithelial connective tissue thereby aiding in secretory and absorptive roles of the epididymis (Adams, 1983), thus serves as a marker for assessment of epididymal function. The highest activity of alkaline phosphatase in the epididymal tail (*cauda epididymis*; Beu *et al*., 2007) enabled us to check the photoperiodic variation in cauda epididymal function. The decreased activity of alkaline phosphatase under SD exposure implies a decreased ability of the epididymis to support spermatozoa maturation in response to such photoperiod. Assessment of fructose concentration in seminal vesicle has been recommended as a marker of secretory activity of seminal vesicle (WHO, 2001), which is highly dependent on circulatory androgen level (Moon *et al*., 1970) as also recorded by us. Decreased concentration of both fructose and sialic acid in seminal vesicle and prostate respectively during SD conditions suggest an inhibitory action of elevated level of melatonin on functional dynamics of ASOs thus establishing a clear role of photoperiod on secretory activity of ASOs that might be driven by expression of MT1R and AR jointly.

Figure 1

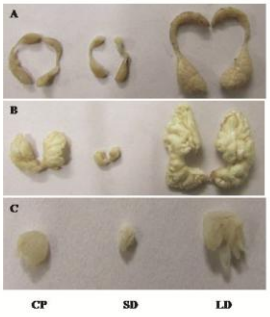

Figure 1

Effect of different photoperiodic conditions (CP, SD and LD) on gross morphology of ASOs. CP = critical photoperiod, SD = short-day photoperiod and LD = long-day photoperiod. A. Cauda epididymis; B. Seminal vesicle and C. Ventral prostate of adult male golden hamster *M. auratus*. Note the regressed morphology of ASOs under SD condition and normal morphological appearence of ASOs exposed to CP or stimulatory LD conditions.

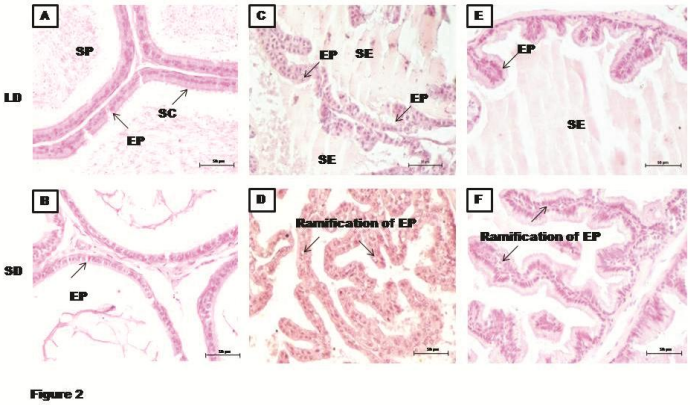

Figure 2

Figure 2
Effect of different photoperiodic conditions (SD and LD) on the histology (haematoxylin and eosin staining) of ASOs. SD = short-day photoperiod and LD = long-day photoperiod. A & B = cauda epididymis; C & D = seminal vesicle and E & F = ventral prostate of adult male golden hamster *M. auratus*. Symbols on illustration indicate SP= Spermatozoa, EP = Epithelium, SC = Stereocilia and SE = Secretion; scale bar = 50µm.

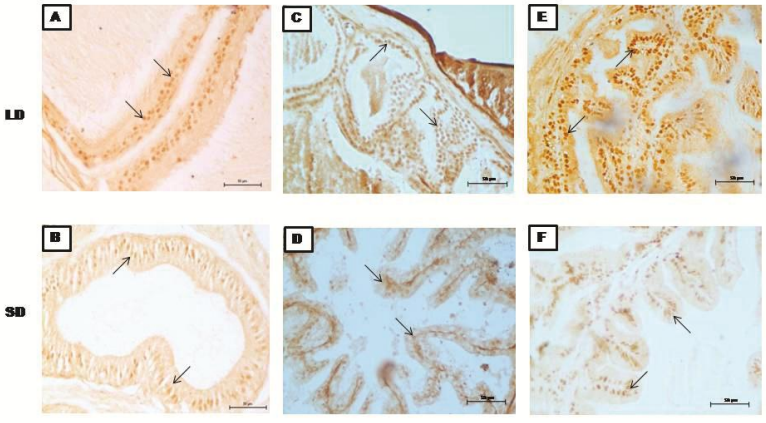

Figure 3

Figure 3
Immunohistochemical localization of androgen receptor in the ASOs exposed to different photoperiodic conditions (SD and LD). SD = short-day photoperiod and LD = long-day photoperiod. A & B = cauda epididymis; C & D = seminal vesicle and E & F = ventral prostate of adult male golden hamster *M. auratus*. Arrows indicate immunoreactivity for AR in the respective tissues, scale bar = 50µm.

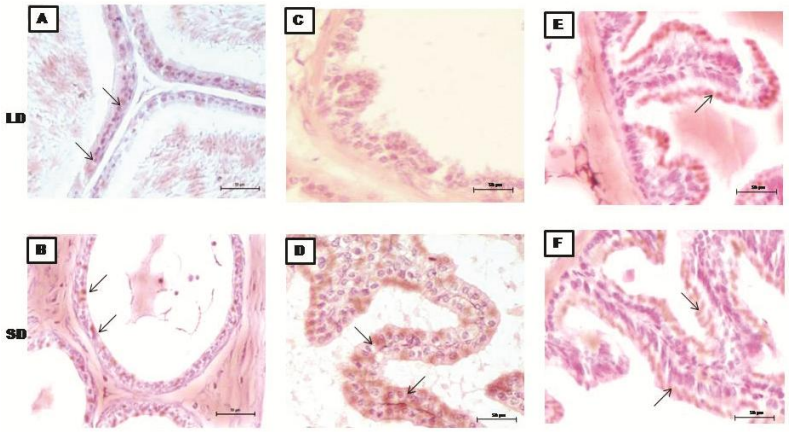

Figure 4

Figure 4
Immunohistochemical localization of MT1R in the ASOs exposed to different photoperiodic conditions (SD and LD). SD = short-day photoperiod and LD = long-day photoperiod. A & B = cauda epididymis; C & D = seminal vesicle and E & F = ventral prostate of adult male golden hamster *M. auratus*. Arrows indicate immunereactivity for MT1R in the respective tissues, scale bar = 50µm.

Figure 5

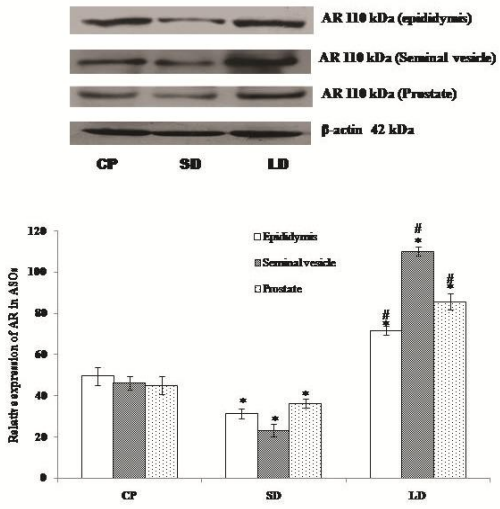

Figure 5

Effect of different photoperiodic conditions (CP, SD and LD) on androgen receptor (AR) expression in ASOs. Western blot analysis of AR in ASOs. CP = critical photoperiod, SD = short-day photoperiod and LD = long-day photoperiod. The data are expressed as relative band intensity of AR expression in ASOs in CP, SD and LD exposed groups of *M. auratus*. β- actin expression was used as loading control. Values are expressed as mean ± SEM, n = 7. Significance of difference *p < 0.05, CP vs. SD and LD and #p < 0.05, SD vs. LD.

Figure 6

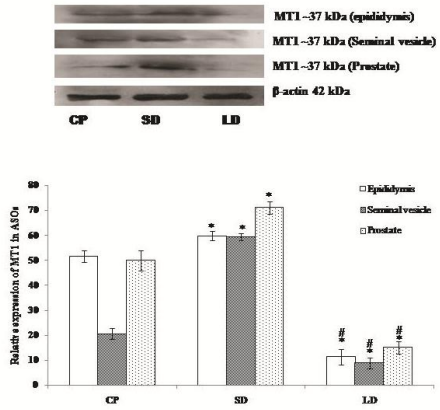

Figure 6

Effect of different photoperiodic conditions (CP, SD and LD) on melatonin receptor (MT1R) expression in ASOs. Western blot analysis of MT1R in ASOs. CP = critical photoperiod, SD = short-day photoperiod and LD = long-day photoperiod. The data are expressed as relative band intensity of MT1R expression in ASOs in CP, SD and LD exposed groups of *M. auratus*. β - actin expression was used as loading control. Values are expressed as mean ± SEM, n = 7. Significance of difference *p < 0.05, CP vs. SD and LD and and #p < 0.05, SD vs. LD.

Figure 7

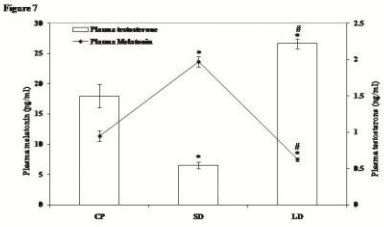

Figure 7
Effect of different photoperiodic conditions (CP, SD and LD) on plasma melatonin and plasma testosterone concentrations. CP = critical photoperiod, SD = short-day photoperiod and LD = long-day photoperiod. Values are expressed as mean ± SEM, n = 7. Significance of difference *p < 0.05, CP vs. SD and LD and #p < 0.05, SD vs. LD.

Table: 1
Effect of different photoperiodic conditions (CP, SD and LD) on weight of ASOs *i.e.* epididymis, seminal vesicle and prostate of adult male golden hamster *M. auratus*. Values are expressed as mean ± SEM; n = 7. Abbreviations: CP = Critical photoperiod; SD= Short day and LD= Long day photoperiod. Significance of difference *$p < 0.05$, CP vs. SD and LD and and #$p < 0.05$, SD vs. LD.

Groups	CP	SD	LD
Cauda epididymis (mg/100 g body weight)	243.33±15.31	67.86±2.63*	318.46±12.41*#
Seminal vesicle (mg/100 g body weight)	622.19±18.25	246.16±7.38*	705.45±15.57*#
Ventral prostate (mg/100 g body weight)	276.93±16.13	60.6±3.76*	341.97±11.63*#

Table 1
Effect of different photoperiodic conditions (CP, SD and LD) on weight of ASOs *i.e.* cauda epididymis, seminal vesicle and prostate of adult male golden hamster *M. auratus*. CP = critical photoperiod, SD = short-day photoperiod and LD = long-day photoperiod. Values are expressed as mean ± SEM; n = 7. Significance of difference *$p < 0.05$, CP vs. SD and LD and and #$p < 0.05$, SD vs. LD.

Table: 2
Effect of different photoperiodic conditions (CP, SD and LD) on alkaline phosphatise activity in cauda epididymis and fructose and sialic acid concentrations in seminal vesicle and ventral prostate. Values are expressed as mean ± SEM, n = 7. Significance of difference *$p < 0.05$, CP vs. SD and LD and and #$p < 0.05$, SD vs. LD.

Groups	CP	SD	LD
Alkaline phosphatase activity (U/ 100 g tissue)	7.67±0.41	2.77±0.24*	10.06±0.35*#
Fructose concentration (µg/100 g tissue)	169.33±8.57	57.07±5.22*	203±10.44*#
Sialic acid concentration (µ moles/ 100 g tissue)	100.87±6.47	42.93±2.54*	138.27±5.85*#

Table 2
Effect of different photoperiodic conditions (CP, SD and LD) on alkaline phosphatise activity in cauda epididymis and fructose and sialic acid concentrations in seminal vesicle and ventral prostate. CP = critical photoperiod, SD = short-day photoperiod and LD = long-day photoperiod. Values are expressed as mean ± SEM, n = 7. Significance of difference *$p < 0.05$, CP vs. SD and LD and and #$p < 0.05$, SD vs. LD.

Conclusion

The present study suggests the existence of a photoperiodically regulated reciprocal interaction between peripheral melatonin and testosterone levels in functionality of ASOs driven by expression pattern of their receptors (AR and MT1R) indicating an intricate physiological mechanism. Such a regulation is of great potential for achieving healthy sperm if and when required that can be procured by exposure to artificial long photoperiod that modifies hormones and its receptors on ASOs.

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