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Biological Significance of Reproductive Phase-Dependent Adjustment of Metabolic, Energetic and Immunologic Adaptations in a Male Seasonal Tropical Breeder *F.Pennanti*: Role of Melatonin and Thyroxin.

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ABSTRACT:

Daily variations in immune parameters lymphocyte (blood and bone marrow), splenic and thymic proliferation in relation with peripheral melatonin (MEL) and thyroxin level (T_4) were measured in a seasonally breeding rodent, Indian palm squirrel, Funambulus pennanti during two reproductive phases i.e. Reproductively Active (RAP) and Inactive phase (RIP). Plasma level of peripheral T_4 reflected its correlation with daily variation of immune status and could be due to its positive role in T cell maturation and reproduction. Regardless of season, all immune parameters examined exhibited a daily rhythm with maximum values during darkness, which coincided with nocturnal increase in peripheral MEL and a decrease in T_4 concentration. Diurnal rhythm in both hormones MEL and T_4 had inverse relation. However, MEL and immune parameters exhibited parallel relation. During RAP the day time mean value of MEL, percent lymphocyte count of bone marrow (BML), blood (BLC) and percent stimulation ratio (% SR) of thymocytes and splenocytes were significantly low with no significant change in daily variation in comparison with those of RIP except T_4, which was significantly high during RAP. The compromised daily variation in immune parameter and MEL during RAP might be compensated with high level of metabolic hormone T_4 for necessary immune requirement, while melatonin takes care of immune variation during RIP.

Keywords: *Melatonin, Thyroxin, Reproductive phase, Tropical rodent*

INTRODUCTION

Each vertebrate species possess a characteristic rhythm of daily activity, being either diurnal (when the mental and locomotor activity as rule are enhanced during the light period of the day while sleep or resting coincides with the period of darkness, like humans) or nocturnal (active during the darkness like mice, hamsters and rats). The pineal gland is responsible for the transformation of external signals, mainly photoperiodic information, into a hormonal output, melatonin in a rhythmical fashion. The cyclicity in pineal melatonin content reveals a consistent phase relationship with cyclical changes in several environmental factors. Immune parameters of man and vertebrates change in the circannual and circadian cycle, including the

number of circulating immunocompetent cells (leukocytes) and their products (e.g. cytokines), which are about 2-3 times greater at a peak compared with the minimum values [1].It has been demonstrated that pineal gland is involved in photoperiodic regulation of endocrine changes including a decrease in circulating level of total and free thyroxin under influence of short photoperiod and blinding [2,3]. Thyroxin being a metabolic hormone is known to be responsible for regulating the phase of reproduction and maintenance of immunity [4,5,6] The inhibitory role of melatonin on the thyroid gland for the secretion of thyroxin has been investigated in several mammalian species[7,4,8].

Daily administration of melatonin for 60 days reduced circulating thyroxin level [9,4,10] indicating the involvement of melatonin in the regulation of thyroid function, but the effect of L-thyroxin on daily variations of immune parameters were never reported. Research till date provided strong evidence for a reciprocal relationship between the immune system and hormones of the hypothalamus-pituitary-thyroid (HPT) axis [11]. In particular, the data on the correlation between daily variations in plasma melatonin and thyroxin level, and those in immune parameters in mammalian species under natural environmental conditions, especially in seasonally breeding tropical rodents are completely lacking.

Thus, the aim of present study was to note the interrelationship between daily variation in pineal and thyroid glands activity (plasma melatonin and thyroxin level) with that of immune status, as judged by the total leukocyte count (TLC) bone marrow lymphocyte (BML) percentage and blastogenic response in terms percent (%) stimulation ratio of thymocytes and splenocytes in this tropical seasonal breeder during its two important reproductive phases i.e. Reproductively Active (RAP) and Inactive (RIP) phase, when this tropical rodent faces drastic environmental changes like photoperiod, temperature and humidity and the environmental threats (seasonal diseases, lack of food and shelter) to their life.

MATERIAL AND METHOD

Animals

The study plan and protocol was approved by the ethical committee of Banaras Hindu University, Varanasi (U.P.). Experiments were conducted according to the guidelines approved by the relevant authorities in India (Indian National Science Academy and Indian Council of Medical Research).

The experiments were conducted on (n= 60; 10 squirrel for each time point) adult male squirrels (body weight~120±5g) during reproductively active (RAP: March; Photoperiod L: D approx. 14L: 10 D; Maximum and Minimum temperature. 37 ± 5^{o} C and 26 ± 5^{o} C, respectively) and inactive phase (RIP: November; Photoperiod: approx. 11L: 13 D; Maximum and Minimum temperature 15 ± 5^{o} C and 6 ± 3^{o} C, respectively). The squirrels were collected from the vicinity of Varanasi (Lat.25^{0}18′ N; Long. 83^{0}1′ E) and acclimatized for two weeks in laboratory condition equivalent with ambient conditions. They were fed with soaked gram and water *ad libitum*. Seasonal fruits and nuts were also provided along with their food.

The optimum care is always taken during the experiments and the sacrifice. The laboratories were maintained with availability of air conditioners, good food, seasonal fruits and

vegetables and water *ad libitum.* The stress elements were avoided inside the animal laboratory.

Sample Collection and parameter studied

Squirrels were selected randomly and sacrificed by decapitation at every four-hour interval starting from 06.00 hours,10 animals in each time-point was used. Blood was collected into heparinized tubes and processed for percent lymphocyte count (%LC). Plasma was separated by centrifugation and was stored at -20^{o} for radioimmunoassay (RIA) of melatonin and thyroxin (T_4). Spleen and thymus were dissected out on ice and processed for lymphocyte (splenocytes and thymocytes) culture. For bone marrow cell collection, femur bones of both legs were dissected out and bone marrow strip was flushed with phosphate buffer solution with the help of syringe into a test tube and agitated to obtain a homogenous suspension.

Hormonal Analysis

The RIA of plasma melatonin concentration was performed following modified method of Rollag and Niswender [12] and for thyroxin commercial kit from T4 RIA; Immunotech, Czech Republic, was used.

Hematological parameters

Blood film was prepared for lymphocyte count of blood and bone marrow (BLC and BML), total leukocyte count was done in Neubauer hemocytometer.

Percent Lymphocyte Count (% LC) of Peripheral Blood and Bone Marrow

For the percent lymphocyte count thin blood and bone marrow cell suspension films were prepared and stained with Leishman's stain and differential leukocyte count was done and documented.

Reagent and culture medium for blastogenic response

Tissue culture medium RPMI-1640 and all other chemicals were purchased from Sigma Chemicals, USA. The culture medium was supplemented with antibiotics (Penicillin 100 IU/ml, streptomycin 100 µg/ml, gentamycin 100 µg/ml) and 10% fetal calf serum. Thymus and spleen were processed for preparation of single cell suspension. The number of cells was adjusted to $1x10^6$ cells / ml in RPMI 1640 complete medium containing sodium bicarbonate. For the study of blastogenic response and percent stimulation ratio of the cell suspension placed in duplicate of cell and cultured for 72 hours in CO_2 incubator with 5 % CO_2 at 37°C. Culture tubes were cultured in the absence of mitogen whereas the test cultures were stimulated with T-cell mitogen concanavalin A (Con A) at the concentration 20µg/ml following the method of Pauley and Sokal [13]. The Blastogenic response and % SR was measured for lymphoid cell proliferation and assayed by pulse labeling with tritiated thymidine uptake (^{3}H–TdR; Specific activity 8.9ci mM; BARC Mumbai, India), 18 hr before the end of incubation period against stimulation by Con A of the thymocyte and splenocyte. A 0.1ml aliquot was counted using a liquid scintillation counter (Packard, USA). Results are expressed as [3H]-TdR incorporation in counts per minute. The percent stimulation ratio was calculated as follows:

$$\% \text{ Stimulation Ratio} = \frac{\text{cpm with mitogen}}{\text{cpm without mitogen}} \times 100$$

Statistical Analysis

Statistical analysis of the data was performed with one-way ANOVA followed by a student Newman Keul's test. The differences were considered significant when $P<0.05$.

RESULT

Daily variation in Plasma Melatonin

Plasma melatonin showed a significant daily change during both Reproductively Active and Inactive Phases. During reproductively active phase, the maximum plasma level was noted at 02:00 hours at night and a minimum plasma melatonin level was noted at 06:00 hours (Figure.1a). During reproductively inactive phase, maximum plasma level of melatonin was noted at 02:00 hours in the night. However, a small peak of plasma melatonin was also noted at 14:00 hours in the day time. (Figure 1a)

Daily variation in Plasma Thyroxin

Plasma thyroxin showed a high basal level during reproductively active phase. The minimum plasma thyroxin was noted at 02:00 hours during both the reproductive phases (Figure.1b). During reproductively inactive phase the basal level of thyroxin was noted without any significant rhythm being maximum at 06:00 hours in the morning time. (Figure.1b)

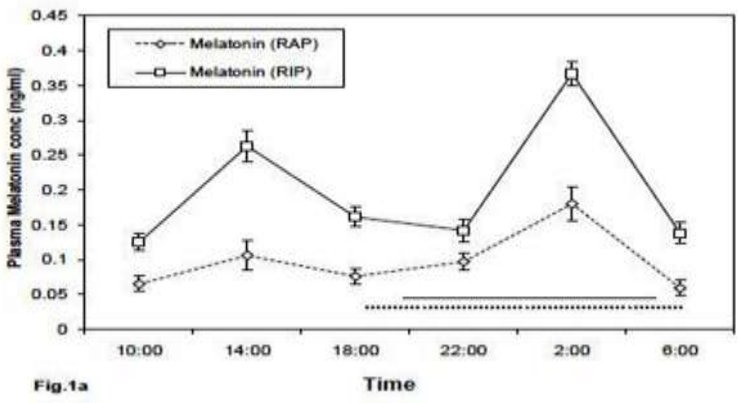

Fig.1a

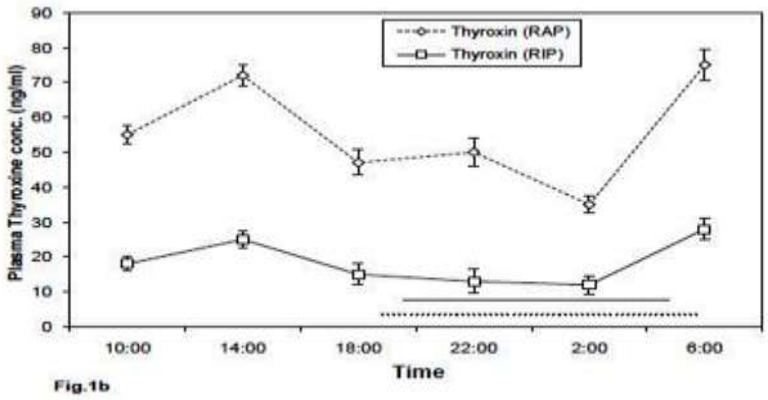

Fig.1b

Fig.1(a,b). Daily variation in plasma melatonin (ng/ml) and plasma thyroxin concentration (ng/ml) of Indian palm squirrel, Funambulus pennanti during reproductively active phase (RAP) and reproductively inactive phase (RIP) Data presents Mean ± SEM. Vertical bar shows standard errors.

Daily variation in Total Leukocyte and Lymphocyte Count

Total Leukocyte Count during reproductively active phase showed maximum count at 02:00 hours and a minimum count for total leukocyte and lymphocyte during reproductively active phase (RAP) was noted at 06:00 hours during the morning time. However during reproductively active phase the total leukocyte count has no significant rhythm (Figure 2a). A significant daily variation was noted in Total leukocyte and lymphocyte count during reproductively inactive phase being maximum at 02:00 hours with greater amplitude of the counts during reproductively active phase. (Figure.2b)

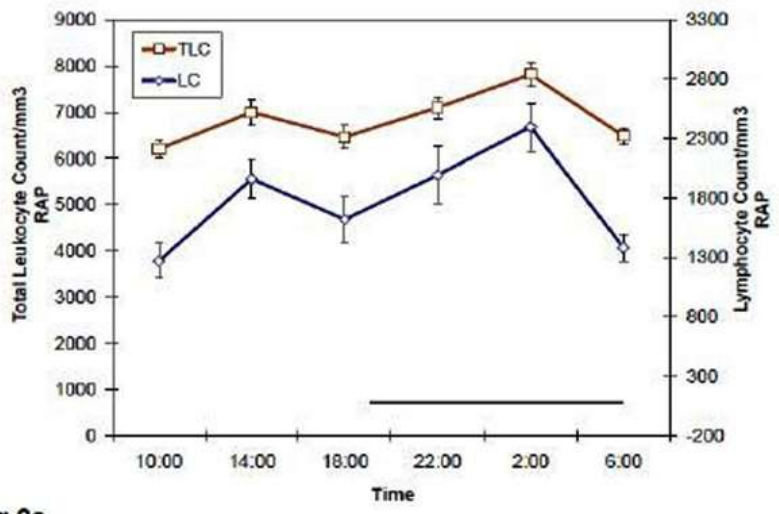

Fig 2a

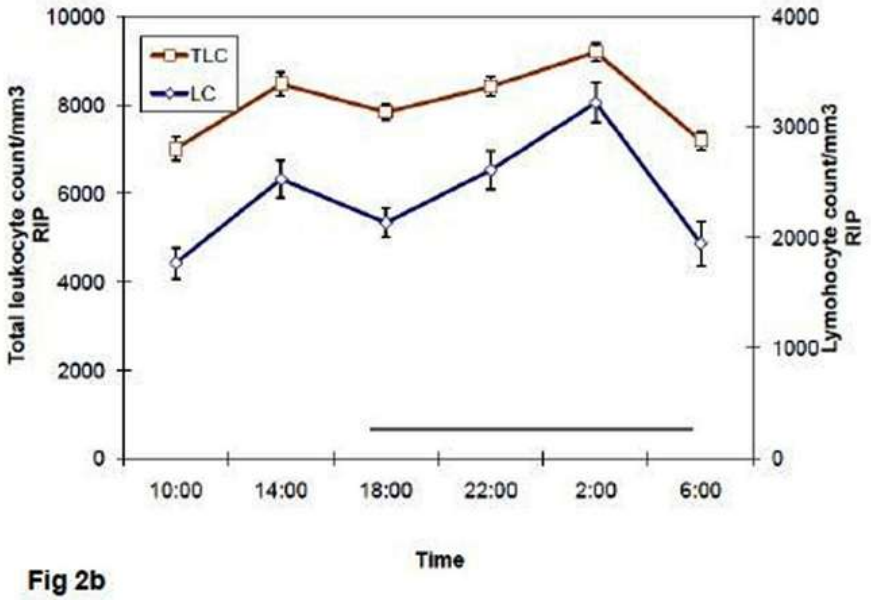

Fig 2b

Fig.2(a,b). Daily variation in total leukocyte count (TLC/mm^3) and lymphocyte count (LC/mm^3) of Indian palm squirrel, Funambulus pennanti during reproductively active phase (RAP) and reproductively inactive phase (RIP). Data presents Mean ± SEM. Vertical bar shows standard errors.

Daily variation in Percent Lymphocyte of Blood (% BLC)

The basal level of percent lymphocyte count showed no significant during both the Reproductive phases. A remarkable rhythm during both the reproductive phases was noted. During reproductively active phase, the maximum percentage of blood lymphocyte count was noted at 02:00 hours (30.6%) and minimum was observed at 06:00 hours (21%) (Figure.3a).

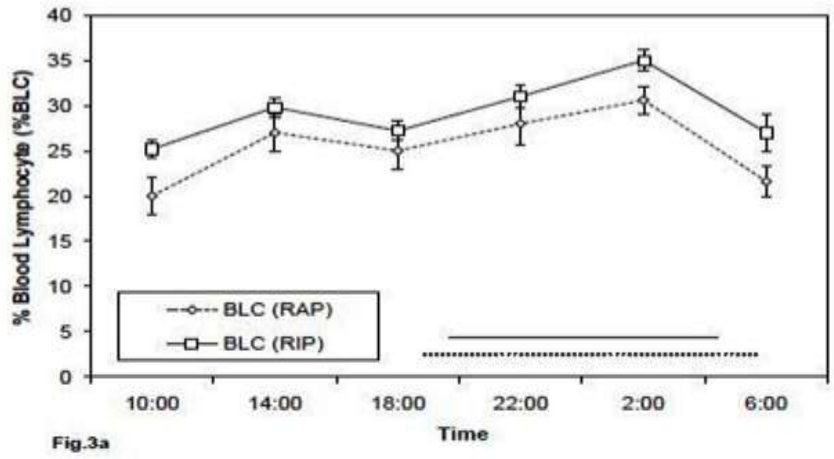

Fig.3a

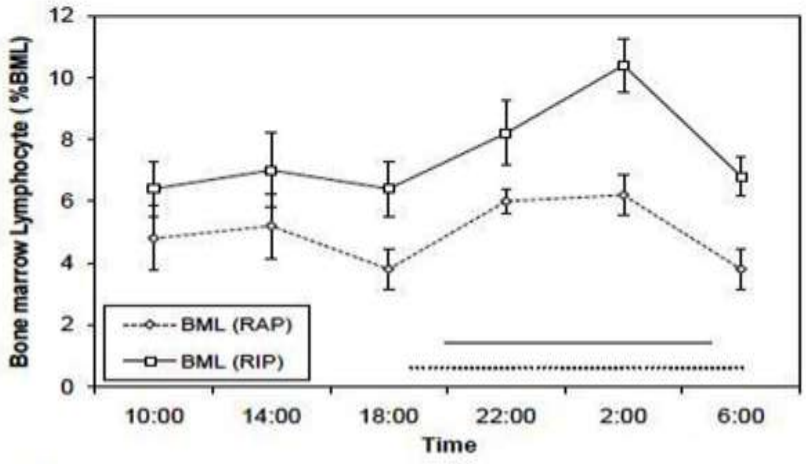

Fig.3b

Fig.3 (a,b). Daily variation in percent lymphocyte count of peripheral blood (% BLC) and bone marrow (% BML) of Indian palm squirrel, Funambulus pennanti during reproductively active phase (RAP) and reproductively inactive phase (RIP). Data presents Mean ± SEM. Vertical bar shows standard errors.

During reproductively inactive phase, a high percentage of blood lymphocyte count was noted at 02:00 hours (35%). A minimum count for percent blood lymphocyte count was observed at 10:00 hours (25.2%). (Figure 3a)

Daily variation in Percent Bone Marrow lymphocyte count (%BML)

The percent count of Bone marrow lymphocyte showed a maximum percentage count at 02:00 hours (6.2%) and minimum count at 06:00 hours (3.8%) during reproductively active

phase (Figure.3b).During reproductively inactive phase, the higher count of percent bone marrow lymphocyte was noted at 02:00 hours (10.4%). However, a minimum count was noted at two time point scale 06:00 hours and 18:00 hours (6.4%). (Figure 3b)

Daily variation in Blastogenic response and Percent Stimulation Ratio (% SR) of Thymocyte

Blastogenic response was observed in the terms of basal as well as T-Cell mitogen Concanavalin A (Con A) induced thymocyte proliferation in culture. Significant daily changes were observed in the blastogenic response of thymocyte during both reproductively active and inactive phases. During reproductively active phase, maximum thymocyte blastogenesis (both basal and mitogen stimulated) was observed at 02:00 hours at night and a minimum was observed during 06:00 hours in the morning (Figure 4a). During the reproductively inactive phase, the maximum thymocyte blastogenesis both basal and mitogen stimulated was observed at 02:00 hours and a minimum was noted at 06:00 hours. (Figure 5a)

Significant daily changes were noted in percentage stimulation ratio of thymocytes during both reproductively inactive and reproductively active phase. During reproductively active phase the percent stimulation rate was noted maximum at 02:00 hours at night and minimum %SR was noted at 10:00 hours (Figure 4b). During reproductively inactive phase the maximum

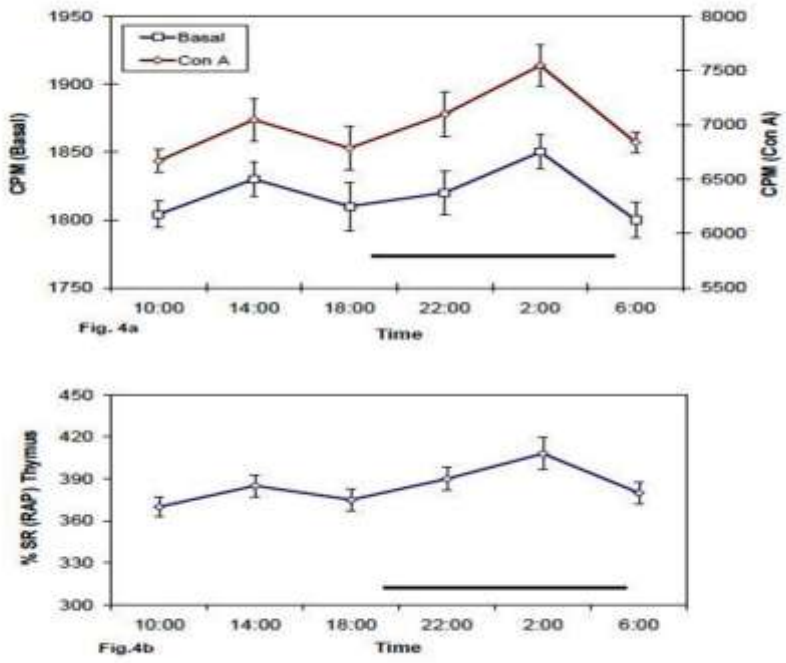

Fig. 4(a,b). Daily variation in basal and mitogen (ConA) induced blastogenic response (a) and percent stimulation ratio (%SR); (b) of thymocytes of Indian palm squirrel, Funambulus pennanti, during reproductively active phase (RAP). Data presents Mean ± SEM. Vertical bar shows standard errors. minimum was observed during 06:00 hours in the morning (Figure 4a). During the reproductively inactive phase, the maximum thymocyte blastogenesis both basal and mitogen

Daily variation in Blastogenic response and Percent Stimulation Ratio (% SR) of Thymocyte

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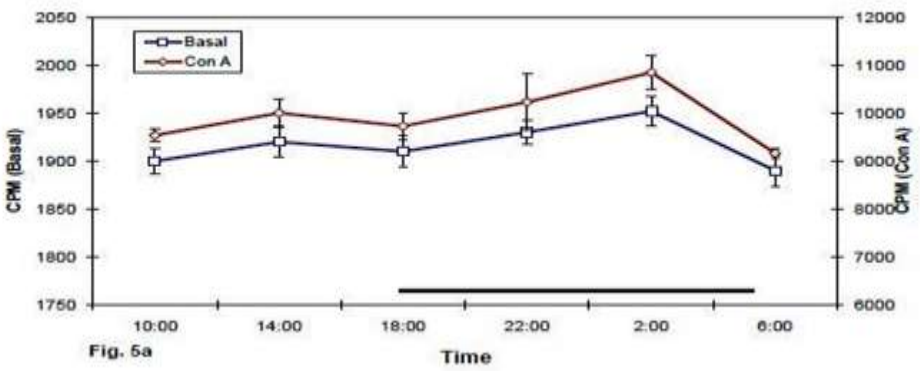

Fig. 5a

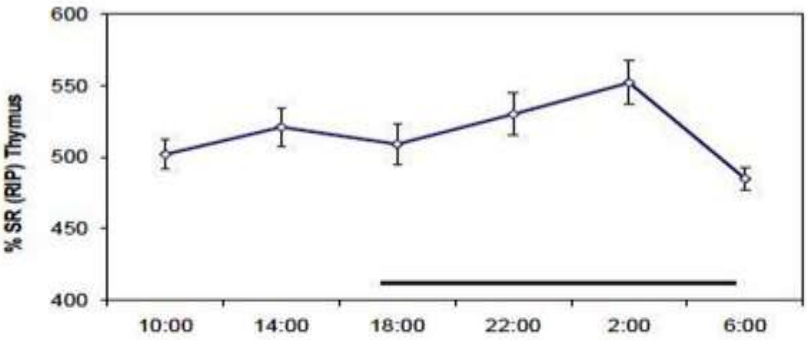

Fig. 5b

Fig. 5(a, b). Daily variation in basal and mitogen (ConA) induced blastogenic response (a)and percent stimulation ratio (%SR); (b) of thymocytes of Indian palm squirrel, Funambulus pennanti, during reproductively inactive phase (RIP). Data presents Mean ± SEM Vertical bar shows standard errors.

During the reproductively inactive phase, the maximum thymocyte blastogenesis both basal and mitogen stimulated was observed at 02:00 hours and a minimum was noted at 06:00 hours. (Figure 5a)

Significant daily changes were noted in percentage stimulation ratio of thymocytes during both reproductively inactive and reproductively active phase. During reproductively active phase the percent stimulation rate was noted maximum at 02:00 hours at night and minimum %SR was noted at 10:00 hours (Figure 4b). During reproductively inactive phase the maximum % SR was noted at 02:00 hours at night and the minimum was noted at 06:00 hours. (Figure 5b)

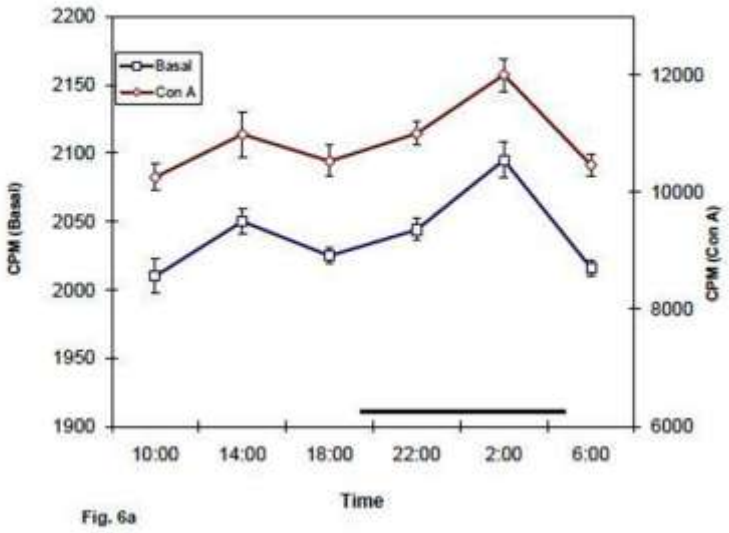

Fig. 6a

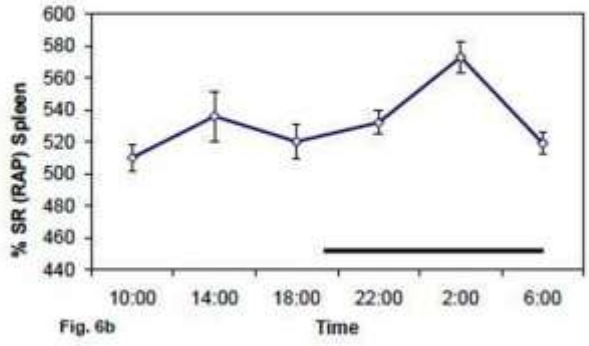

Fig. 6b

Fig. 6(a). Daily variation in basal and mitogen (ConA) induced blastogenic response and percent stimulation ratio (% SR) of splenocytes of Indian palm squirrel, Funambulus pennanti during reproductively active phase (RAP). Data presents Mean ± SEM). Vertical bar shows standard errors.

Daily variation in Blastogenic response and Percent Stimulation Ratio (% SR) of Splenocyte

Blastogenic response was observed in the terms of basal as well as T-Cell mitogen Concanavalin A (Con A) induced thymocyte proliferation in culture. Significant daily changes were observed in the blastogenic response of thymocyte during both reproductively active and inactive phases. During reproductively active phase, maximum splenocyte

blastogenesis (both basal and mitogen stimulated) was observed at 02:00 hours at night and a minimum was observed during 06:00 hours in the morning (Figure 6a). During the reproductively inactive phase, the maximum splenocyte blastogenesis both basal and mitogen stimulated was observed at 02:00 hours and a minimum was noted at 06:00 hours. (Figure 7a)

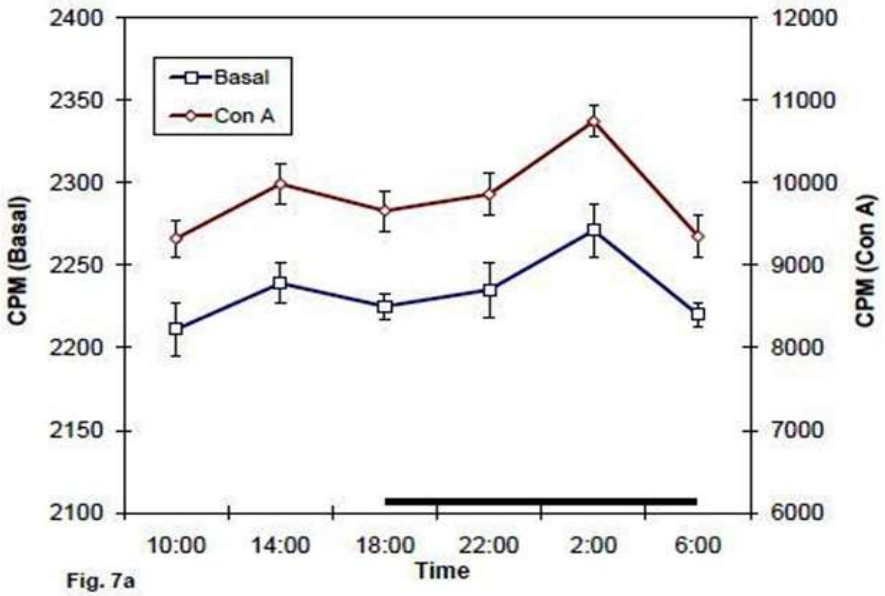

Fig. 7a

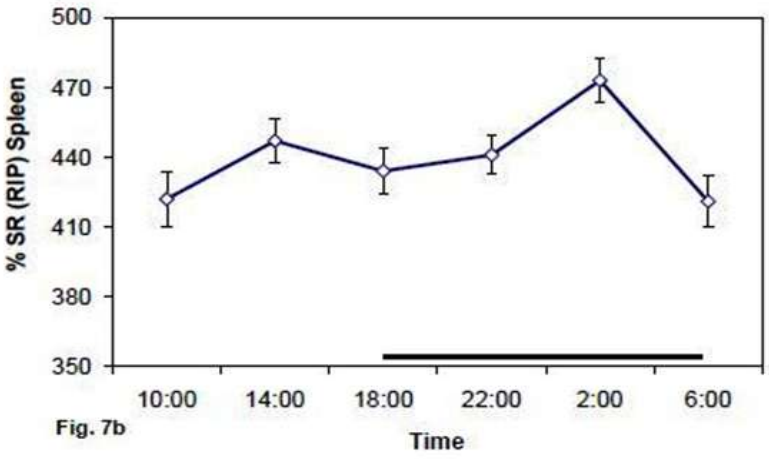

Fig. 7b

Fig. 7(a). Daily variation in basal and mitogen (ConA) induced blastogenic response (a) and percent stimulation ratio (% SR) of splenocytes of Indian palm squirrel, Funambulus pennanti during reproductively inactive phase (RIP). Data presents Mean ± SEM). Vertical bar shows standard errors.

Significant daily changes were noted in percentage stimulation ratio of splenocytes during both reproductively inactive and reproductively active phase. During reproductively active phase the percent stimulation ratio was noted maximum at 02:00 hours at night and minimum

%SR was noted at 10:00 hours (Figure 6b). During reproductively inactive phase the maximum % SR was noted at 02:00 hours at night and the minimum was noted at 06:00 hours. (Figure 7b).

DISCUSSION:

The role of pineal in controlling behavioral and activity rhythms as already being established [14], further the rhythmic synthesis and secretion of the pineal hormone melatonin is suggested to be the mechanism by which pineal controls circadian oscillator(s) elsewhere for various other functional rhythms [15]. The immune parameters peak at one time of the day for a diurnal species, peak about 12 hours later for a nocturnal species. [16] However, Nelson and Demas 2004, [17] has reported that various immune parameters peak at various time points, anticipating an encounter with a pathogen during the period of activity while undergoing energetically expensive resolution of the immune response during the resting period. The daily and seasonal cyclicity of the immune function are partially integrated with the other behavioral and physiological processes and all of them are regulated and coordinated with daily and seasonal changes of an external environment by the neuroendocrine homeostatic system [18] which proves once more the biological significance of the rhythms in immunity for survival.

The present study first time bringing a daily variation in melatonin secretion and metabolic hormone thyroxin alongwith the daily variation in immune status (TLC, LC, BLC, BM and %SR of a seasonally breeding tropical rodent *Funambulus pennanti*) . The experiment on daily variation was performed during the two reproductive phases of this rodent i.e., Active and Inactive phase when not only the metabolic status but the environmental factors such as Photoperiod and temperature as well present drastic changes which may in turn influence the neuroendocrine axis including pineal activity. During these two reproductive phases the natural photoperiod and temperature conditions were completely different and hence the melatonin peak was at least 4 hours advance (Phase advanced) during the reproductively inactive phase than during the active phase. This shift in peak phase of melatonin level could be due to decrease length of day time (Photoperiod in the month of January in comparison with the month of June) [19]. Available literature suggest that pattern of nocturnal pineal production of melatonin varies with the species [20] i.e., the peak may occur early during the dark period, near the middle of the dark period or throughout the dark period [21]. Interestingly slightly high melatonin levels were noted at 14:00 hours due to high tropical afternoon temperature leading to afternoon *siestas* and energy balance [22] which decreases again at 18:00 hours during the winter and reaches that peak at midnight. Such kind of variation was not observed in summer as the basal level of the melatonin was already low (Figure 1a). Bubenik and his group evidenced that the Gastrointestinal Tract (GIT) acts as a reservoir of melatonin and activates the digestive processes during afternoon [23]. GIT is also known as a significant contributor of melatonin to the peripheral circulating levels [24]. In the present observation a sudden elevation in melatonin concentrations in Blood was noted by us, which may account for hypothalamic condition [25] leading to the sleepiness after meal and *siestas* [26]. It is also reported by Bubenik in 2002 that due to the hypothermic effect siesta is most popular in hot, periequitorial region of the earth as in the cases of tropical animals. [26]

Our animal model *F. pennanti* is a seasonal breeder of tropical Indian origin. The reproductive activity of this rodent starts in the end of January and peaks in March and extends to August. Reproductive inactivity commences between September and December and is least in November. The pineal gonadal interrelationship during the year has been studied extensively and a complete inverse relationship has been documented [27] (Rai and Haldar 2006 JER)**.** Further, the interrelationship between pineal gland and melatonin with that of thyroid hormone and immune functions had been very established and published elsewhere [28, 29].

Melatonin (N-acetyl-5-methoxytryptamine), the main indoleamine secreted by the pineal, exhibits a circadian rhythm that conveys environmental information, particularly about photoperiod to the organism [6]. Our work presents a daily variation in pineal and thyroid gland activity (melatonin and thyroxin secretion) along with daily variations in the immune status (LC, % lymphocyte of peripheral blood and bone marrow and % SR of spleen and thymus) of a seasonally breeding tropical rodent. The correlation of immunity and melatonin in response to daily variations and photoperiod is being reported for the first time in this tropical rodent. In contrast to the cyclicity of melatonin and other hormones several immune parameters correlates with the pattern of the animal's daily activity. It can be anticipated that melatonin acts on immune cells at several levels as the melatonin receptors are present on the lymphoid organs as well as on lymphocytes suggest that melatonin plays a key role in immunomodulation [29].

We have noted an inverse relationship between circulating melatonin and thyroxin level of this tropical rodent [6]. Further, an inhibitory effect of melatonin on circulating T_4 level was also noted in this tropical rodent. The control of melatonin in the generation of daily variation of circulating T_4 level might be at the hypothalamus-pituitary level as receptor for melatonin was noted on pars tuberalis and on certain hypothalamic region which may alter the secretion of TSH. Melatonin may affect directly at the level of thyroid gland as receptor for melatonin was detected on thyroid gland itself [30]. Thyroid stimulating hormone (TSH), in particular, has been shown to have a variety of immune-regulating cytokine-like activities that can influence the outcome of T-cell development in the thymus and intestine, and can affect the magnitude of antibody and cell-mediated responses of peripheral lymphocytes. Production of TSH and the expression of the TSH receptor are widely but selectively distributed across many different types of hematopoietic cells in the bone marrow, as well as among subsets of dendritic cells, monocytes and lymphocytes in the spleen and lymph nodes [31]. Further, thyroxin hormone enhances immune function [32] by promoting thymocyte maturation and differentiation [33, 34]. The thyroid gland and its hormones have been reported to influence reproduction [35, 36] metabolism [37] and the immune status of animal [10, 38] have reported an inhibitory effect of melatonin on the thyroxin in *in vivo* as well as *in vitro*.
Our study suggest that even in tropical countries, where day length are having not much difference in summer and winter (RAP and RIP) a diurnal rodent however, exhibit significant daily variation in immune parameters and the hormones which are involved in regulation of immune parameters. We have published already that peripheral melatonin concentration of our animal model presents daily variation which is very much depend not only on the seasonal changes (photoperiod, temperature, and humidity) but dependent on the reproductive phase i.e. dependent on gonadal hormone [6]. Variation of blood lymphocyte count (BLC)

during RAP and RIP is not very significant while the bone marrow lymphocyte (BML) of RIP and RAP appears to be photoperiodic sensitive being more dependent on reproductive phase or internal gonadal steroid and melatonin. Similarly there was no significant variation in % SR of splenocytes and thymocytes except that the percentage was much higher for both the immune cell during RIP than RAP.

Diurnal rodents are being metabolically active during day time and knowing the importance of thyroxin in regulation of basal metabolic rate (BMR) and intermediary metabolism and also its importance in regulation of reproduction we thought to note its rhythmicity in relation with immunity and melatonin. Melatonin has been accepted as a chronobiotic molecule but thyroxin was never. Interestingly we found that day time high thyroxin at 14.00 hours coincided with the slightly high melatonin, blood lymphocyte and percent stimulation ratio of splenocytes while it has strong inverse relation with melatonin at 2.00 hours which coincided with % SR of splenocytes, thymocytes,% BLC, and % BML. This phenomena was quite interesting for us leading to suggest that when BMR of the squirrels were low during night, there energy requirement was less, the reproductive activity is low, the immunity and melatonin becomes high. Hence, energy balance is important equally in day time when steroids and T_4 works for high energy requirement while during night it is spend for immune status. It is also to be noted that trade off relationship thought to occur at a gross range i.e. reproductive functions/ phases. Rhythmic expression of hormone which are associated with immunity might be also required for resetting the daily physiological rhythm of immunity, slowly for each season and reproductive phase so that the survival is maximum.

In view of the variety of possible direct effects of melatonin on intracellular signaling (the existence of different type of melatonin receptors) and the fact that lymphocytes themselves may produce melatonin in response to certain stimuli, melatonin, in addition to its endocrine role, can be expected to have a physiological impact as a paracrine or even intracellular modulator within immune system. It was interesting to note that the two endocrine glands, the thyroid and pineal (affecting the hematological parameters for our immune system) have an innervations originating from a common superior cervical ganglion. These organs hence might be utilizing single neurotransmitters (melatonin) for signal transduction. Further, the availability of hormones receptors on each gland like, melatonin receptors on thyroid; T_4 receptors on pineal led us to suggest the interdependency in immune regulation and functional communication between them for a better adaptive significance in the proposed animal model [39].

During reproductively inactive phase short photoperiod induced melatonin secretion acting as a blaster for the immune status in the winter phase to help the squirrel to combat with seasonal stressor (low temperature, lack of food, shelter) that would otherwise compromise immune function to critical level while, during reproductively active phase steroid hormone like thyroxin and also other eco-factors (high temperature, long photoperiod and amount availability of food) helped the tropical rodent to remain healthy. The tropical rodent presented a strong interrelationship and interdependency between pineal-thyroid reproductive functions [38]. Peripheral thyroxin in (daytime) and melatonin (night-time) showed a seasonal variation having almost an inverse relationship [40]. Further, interdependency of

pineal-thyroid in relation with thymus of Indian tropical rodent was also proposed by our lab [41].

Considering the effect of reproductive phase dependent melatonin concentration in the release of thyroxin it could be that elevated melatonin amplitude and mesor [6] might have acted negatively on the hypothalamus-hypophyseal –thyroidal axis by inhibiting the thyrotropin releasing hormone or on the thyroid stimulating hormone or mitotic activity of thymocytes and finally altered the rhythmic character of thyroxin by lowering mesor and amplitude [29].

We have noted an interesting feature of the functional relationship of thyroxin and melatonin and suggested a "Trade off" hypothesis between these two hormones[10], which justifies the statement that these two hormones together had no additive response, while in dissociation they promoted the immune function during two different reproductive phases for benefit of survival of animals. Therefore, it may be said that pineal gland and its hormone melatonin acts like a major temporal synchronizer to maintain a humoral and immune adaptability of this tropical rodent in a rhythmic manner. The augmentation of thyroxin function by melatonin is not clearly known. However, melatonin receptors have also been detected on the circulating lymphocytes [42] as well as thymocytes and splenocytes [43] may be site of melatonin action through which melatonin synergizes the thyroxin modulated immune functions.

Our results suggest that the biological significance of this rodent may be correlated on one hand with the rhythm of immune parameter on the other hand it can also be directly correlated with circulating melatonin level which acts like a major temporal synchronizer to maintain the hormonal and immune adaptability in this rodent.

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