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Effects of photo stimulation and nonstimulation of golden hamsters (*Mesocricetus auratus)* from birth to early puberty on testes structure and function

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Summary. We tested whether puberty in golden hamsters is photoperiodically controlled. Hamsters were raised under 14:10 hours Light:Dark (14L) and 1:23 hours Light:Dark (1L) respectively, from birth to 28 days and tested for various parameters. Body weight, Leydig cell (LC) size and testicular testosterone secretion were greater and plasma thyroxin (T4), testicular androstenedione secretion and LC number were lower ($P<0.05$) in 1L than 14L hamsters. Volumes of testicular components were similar in the two groups. 3ß-hydroxy steroid dehydrogenase immunohistochemistry demonstrated LC progenitors and newly formed adult LC (ALC) in 14L hamsters, which were absent in 1L hamsters; they contained only fetal LC (FLC). Latter findings suggest the presence and absence of postnatally-differerentiated LC in 14L and 1L hamsters, respectively. Androgen results agreed with these findings, because FLC primarily secrete testosterone, and androstenedione is a major androgen secreted by the newly formed ALC. Reduced T4 in 1L hamsters is attributed to the inhibition of thyroid function by the increased duration of melatonin secretion due to non-photostimulatory conditions. The arrest in LC differentiation in 1L hamsters is attributed to low T4 levels. Although the testis size is unaltered under non-photostimulatory conditions, postnatal LC differentiation is inhibited in golden hamsters, and therefore, it is logical to suggest that their puberty is photoperiodically controlled.

Key words: Photoperiod, Leydig cell differentiation, Hamster, Testosterone, Thyroid Hormones, Melatonin

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Introduction

Leydig cell differentiation in the postnatal mammalian testis has been well documented in the rat (Mendis-Handagama and Ariyaratne, 2001) and mouse (O'Shaughnessy et al., 2005). This process is described with five different cell types in the lineage (Mendis-Handagama and Ariyaratne, 2001): Leydig stem/precursor cells, Leydig cell progenitors, newly formed adult Leydig cells, immature adult Leydig cells and mature adult Leydig cells.

Information on prepubertal or early pubertal Leydig cell differentiation in the hamster is sparse. However, it is known that in seasonal breeding mammalian males, including the hamster, testes undergo a marked atrophy during non-breeding seasons (Bartke et al., 1996). Under short day lengths, hamster testes show a reduced capacity to secrete testosterone, Leydig cell atrophy and reduction in number, and a reduction of the volumes of Leydig cell organelles associated with steroidogenesis (Bartke et al., 1996); these changes are reversed with exposure to long days (Bartke et al., 1996).

The observations on the effects of photoperiod on adult hamster Leydig cells are suggestive that light is an important factor in maintaining the hormone secretory capacity of these cells. This concept prompts whether light has a regulatory role in the process of Leydig cell differentiation in the developing postnatal testes, especially in seasonal breeders. However, to date, the role of photoperiod on Leydig cell differentiation in the postnatal testis is unclear. Based on the weekly measurements of gonadal and accessory sex organ weights, Darrow et al. (1980) have reported that the onset of puberty in the golden hamster is not photoperiodically controlled. Their information came from two experiments. The first study used hamsters from birth to seven weeks of age and the second study used hamsters blinded from birth to 13 weeks; in both

experiments, these hamsters were subjected to different photoperiodic regimes, which are photostimulatory and non stimulatory. i.e. 14:10 LD, 6:18 LD and 1:23 LD. Their (Darrow et al., 1980) conclusion was that "puberty in the golden hamster is not photoperiodically controlled", which was based solely on the weights of testis and accessory sex glands. Similar conclusions have been made in several other reports on golden hamsters (Gaston and Menaker, 1967; Reiter et al., 1970; Rollag et al., 1982; Sisk and Turek, 1983, 1987; Beery et al., 2008). However, it is important to know whether any structural and functional changes occur in testes of golden hamsters that are subjected to photo non-stimulatory conditions to justify that conclusion.

In the present study, two groups of Golden hamsters, one raised under photostimulatory (14L) and non-photostimulatory (1L) conditions and their testis structure and function were investigated. Findings revealed that postnatal differentiation of Leydig cells in the golden hamster is arrested under the tested photo non-stimulatory condition, which is identical to that in the study of Darrow et al. (1980). However, the present study revealed that circulating levels of testosterone in these hamsters are maintained and could be attributed to the presence of fetal Leydig cells in their testes, which are not regressed during the tested experimental period, and explained the prevention of atrophy of gonads (Gaston and Menaker, 1967; Reiter et al., 1970a,b; Rollag et al., 1982) and accessory sex glands as previously published (Gaston and Menaker, 1967; Rollag et al., 1982).

Materials and methods

Animals

Female and male adult golden hamsters (*Mesocricetus auratus*) were purchased from Charles River Laboratories (Wilmington, MA). They were paired 1:1 (female:male) for breeding in the animal facility of The University of Tennessee College of Veterinary Medicine, and provided food and water *ad libitum*. The approved animal protocol #1366 was used for breeding hamsters.

Experimental Design

The animal protocol #811 was used for the rest of this study. Pregnant female hamsters were divided into two groups. One group was raised under a photostimulatory condition (14:10 hr Light:Dark) and their pups (14L) were raised from birth to 28 days under a similar light regime. The other group of pregnant hamsters were kept under a photo non-stimulatory 1:23 hr, Light:Dark (1:23 LD) regime from one week prior to delivery of pups and their pups (1L) were raised from birth to 28 days under 1:23 LD. Pups of both groups were with their mothers until weaned at postnatal day 21, and raised on food and water *ad libitum* until sacrificed on postnatal day 28. Pups were separated by sex at weaning.

Harvesting blood and preparation of plasma for radioimmunoassays

Only the male hamsters were used in the study. Heparin (Heparin solution for injection, USP, 10,000 units/ml, Steris Laboratory Inc. AZ; 10 U/kg. body weight) was injected 20 minutes prior to euthanasia of hamsters, which was achieved by overdose of inhalation of Isoflurane (Abbott Laboratories, Deerpark, IL). Blood was collected by cardiocenteces, plasma was prepared and stored at -80°C until radioimmunoassays were performed for testosterone, androstenedione and thyroxin (T4).

Tissue Harvesting

One testis from each animal (n=8 hamsters/group) was removed from euthanized hamsters, freed from the epididymis and weighed to obtain the fresh testicular weight, which is required to determine the fresh testis volume to be used in the stereological studies. This testis was used to determine the LH-stimulated testicular androgen (testosterone and androstenedione) secretory capacity in vitro as described previously (Mendis-Handagama et al., 1990, 1998; Ariyaratne and Mendis-Handagama, 2000). The other testis of five of the eight hamsters in each experimental group was fixed in-situ by vascular perfusion with 2.5% gluteraldehyde solution in 0.1M cacodylate buffer (pH 7.4) as described previously (Mendis-Handagama et al., 1988, 1998; Ariyaratne and Mendis-Handagama, 2000). Fixed testes were weighed, cut into approximately 2-3 mm^3 cubes, post fixed in a 1:1 mixture of 2% aqueous osmium tetroxide and 3% potassium ferrocyanide (Russell and Burguet, 1977), dehydrated in graded ethanols and embedded in epon araldite (Mendis-Handagama et al. 1988, 1998). Shrinkage of testis tissue was measured as described previously by Mendis-Handagama and Ewing (1990). The other testis of the other three hamsters was fixed in Bouin's solution, processed and embedded in paraffin to perform immunohistochemistry for 3ß-HSD as described previously (Mendis-Handagama et al., 1998; Ariyaratne and Mendis-Handagama, 2000).

Light microscopy and stereology

From the polymerized blocks of testis tissue, 1μm thick sections were cut using a LKB ultramicrotome V and glass knives. They were picked up onto pre-cleaned glass slides, stained with Methylene Blue Azure II stain and coverslipped under Permount (Fisher Scientific company, Fair Lawn, NJ).

Absolute volumes of leydig cells

Leydig cells in the testis interstitium were identified

under an Olympus BH-2 laboratory microscope (Tokyo, Japan) as described (Mendis-Handagama et al., 1988). Fetal Leydig cells were in clusters, surrounded by basement membrane components. They were large polygonal cells with abundance of cytoplasmic lipid droplets in them. The adult Leydig cells at 28 days were much smaller in size compared to the fetal Leydig cells at this age, were polygonal in shape, had little or no cytoplasmic lipid in them, were not in clusters and did not contain associated basement membrane components. In the present study, the term "Leydig cells" included all types of Leydig cells (both fetal and adult types) but excluded the mesenchymal cells. The volume density of Leydig cells (defined as the volume of Leydig cells per unit volume of testis tissue, expressed as a percentage) were obtained by point counting method (Weibel, 1969) as described (Mendis-Handagama et al., 1988) using a x40 objective, x10 ocular and grid with 121 test points fitted to the ocular lens of the microscope (20 field/block, 10 blocks/animal, 5 animals/group). The absolute volume (mm^3) of Leydig cells per testis was calculated by multiplying the volume density of each component by the fresh testis volume (Mendis-Handagama et al., 1988). As the specific gravity of the testis is approximately 1, the values of testis weight in grams were taken as values for testis volumes in cm^3.

Leydig cell number per testis

The numerical density (Nv) of Leydig cells (defined as the number of Leydig cells per unit volume of the testis tissue) was determined by the Disector method (Sterio, 1984) as described by Mendis-Handagama and Ewing (1990) with modifications as published previously (Ariyaratne and Mendis-Handagama, 2000). Twenty to 30 areas per block and 10 blocks per animal were scored. The total number of Leydig cells per testis was calculated by multiplying the numerical density of Leydig cells by the fresh testis volume (Mendis-Handagama et al., 1988; Ariyaratne and Mendis-Handagama, 2000).

LH-stimulated testicular androgen production in vitro

Each fresh testis removed from each hamster was weighed, decapsulated and incubated in 2 ml of Kreb-Ringer bicarbonate solution (pH-7.4) supplemented with glucose (0.004g/ml) and LH (100 ng/ml; Mendis-Handagama et al., 1988, 1998). These incubations were performed in 20 ml scintillation vials at 34°C in an oscillating water bath (90 oscillations /minute). At the end of 3 hours, the incubation medium was collected, centrifuged at 3000 g for 10 minutes, and the supernatant was separated and stored at -80°C until further analysis.

Radioimmunoassay of hormones

Hormonal assays were performed using commercially available kits, validated for use in hamsters. The assays were run for three times for each hormone to assure reproducibility and to assess inter-assay variability. Each sample was run in duplicate to estimate intra-assay variability. Testosterone in testis incubation media and plasma T4 levels were determined by Coat-A-Count RIA kits (DPC, Los Angeles, CA). Androstenedione levels in testis incubation media were determined by RIA kits from ImmuChem, ICN Pharmaceuticals (Costa Mesa, CA). The inter-assay coefficients of variation for testosterone and androstenedione were 6% and 8%, respectively, and 9% for T4. The intra-assay coefficients of variation ranged from 5-7% for all hormones tested.

Immunohistochemistry for 3ß-hydroxysteroid dehydrogenase (3ß-HSD)

Five micron thick testis tissue sections were cut, de-waxed with xylene and rehydrated with decreasing concentrations of ethanol and brought to deionized water. They were washed in phosphate buffered saline (PBS, pH 7.3) for 5 minutes and then incubated in 3% hydrogen peroxide for 20 minutes. After incubation, sections were washed in PBS and normal goat serum (1%) was added to tissues overnight (4°C) to bind nonspecific proteins. Rabbit polyclonal anti-3ß-HSD was prepared in Dr. Mason's (a coauthor in this paper) laboratory in United Kingdom, and was used at a dilution of 1:2000. Control incubations were carried out using pre-immune serum.

The polyclonal antibody against 3ßHSD was a rabbit IgG antibody against purified human placental 3ß-HSD (Lorence et al., 1990) and has previously been used for immunolocalization of 3ß-HSD antigen in rat testis in many studies including LC differentiation studies in rats (Ariyaratne and Mendis-Handagama, 2000; Majdic et al., 1998; Ariyaratne et al., 2000a-d) and has been validated by Dr. Mason and colleagues to be used in golden hamsters and several other rodent species including the laboratory mouse (J.I. Mason, personal communication). 3ßHSD in testis tissue was detected using a commercially available biotin-streptavidin kit (Avidin-Biotin Complex/ABC, BioGenex, San Ramon, CA) according to the manufacturer's protocol. Sections were counterstained with Harris' hematoxylin, dehydrated with increasing concentrations of ethanol, brought to xylene and cover-slipped using Permount.

Statistics

PC SAS was used for statistical analysis. Differences between the means in the two experimental groups were determined using unpaired t-test. P values of 0.05 or less was considered to be significant.

Results

Table 1 shows comparison of body weights, testis

weights, and volumes of testicular components per testis in 14L and 1L hamsters; except for the increase in body weight in 1L hamsters, significant differences were not observed in the two experimental groups for any other parameters tested. During harvesting of tissue, it was observed that 1L hamsters had abundance of body fat compared to 14L hamsters.

Table 2 shows comparison of LH-stimulated testicular testosterone and androstenedione secretory capacity in vitro, plasma T4, Leydig cell number per testis and average volume/size of a Leydig cell in the two experimental groups. LH-stimulated testicular testosterone secretory capacity in vitro and average volume/size of a Leydig cell were significantly higher and LH- stimulated testicular androstenedione secretory capacity in vitro, plasma T4 levels and Leydig cell number per testis were significantly lower in 1L hamsters compared to 14L hamsters.

Figure 1 shows representative light micrographs from 14L (A) and 1L (B) hamster testes immunolabeled for 3ß-HSD, a marker for steroid secreting cells, including cells in the Leydig cell lineage (16-19), except for mesenchymal precursor cells. Spindle-shaped cells in the peritubular region, which contained the 3ß-HSD label in14L hamsters were identified as Leydig progenitor cells (Fig. 1A, 21-24). Spindle-shaped cells positive for 3ß-HSD were not observed anywhere other than the peritubular region in testes of 14L hamsters. The small round cells in the central region of the testis interstitium of 14L hamsters, which were positive for the

Table 1. Body weights (g), Testis Weights (g) and Absolute Volumes of Components per Testis (mm^3).

Parameter	14L Hamsters (14hr:10hr Light:Dark)	1L Hamsters (1hr:23hr Light:Dark)
Body Weight (g)	64±0.01	74±0.2*
Testis Weight (g)	355±10	370±20
Seminiferous Tubules (mm^3)	292±76	323±45
Leydig Cells (mm^3)	3.9±0.99	3.47±0.87
Macrophages (mm^3)	1.5±0.5	1.7±0.6
Mesenchymal cells (mm^3) (peritubular)	3.1±0.5	2.9±0.4
Mesenchymal Cells (mm^3) (other)	4.1±0.9	3.9±0.8
Myoid Cells (mm^3)	1.0±0.3	1.0±0.1
Blood Vessels (mm^3)	13.6±1.7	14.3±1.4
Lymphatic Space (mm^3)	27.1±7.3	26.2±3.5

Mean±SE. An asterisk (*) depicts a greater value ($P<0.05$) between the means of each component tested in 14L and 1L hamsters.

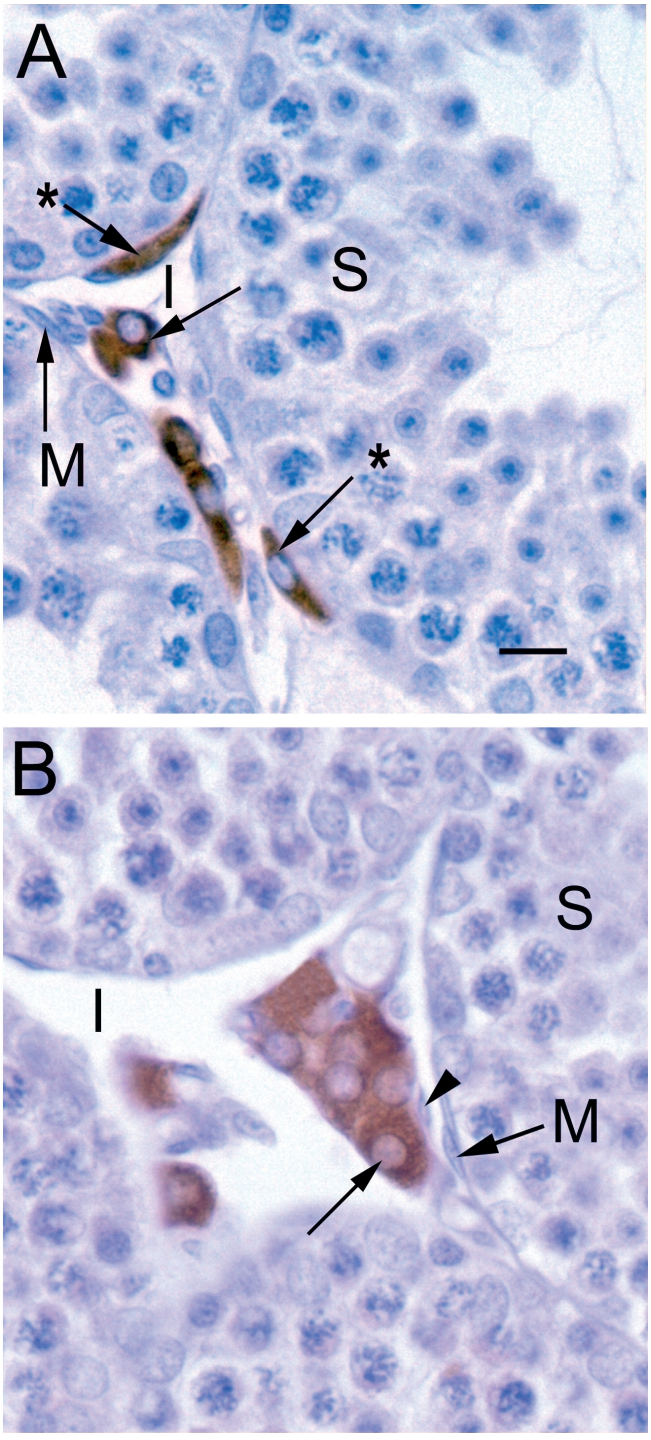

Fig. 1. Representative light micrographs of testes of 14L **(A)** and 1L hamsters **(B)** immunolabeled for 3ß-HSD. S=seminiferous tubules, I=testis interstitium, Bar=20μm for both A and B; same magnification). **A.** Single arrow depicts a newly formed adult LC positive for 3ß-HSD in a 14L hamster testis. Arrows with asterisks (*) depict Leydig progenitor cells, which are spindle-shaped cells located at the peritubular region and are positive for 3ß-HSD. Their presence indicates that LC differentiation is occurring in testes of 14L hamsters. Leydig stem cells/mesenchymal cells (M) are negative for 3ß-HSD. **B.** Single arrow depicts a FLC in a 1L hamster testis. They are primarily observed in clusters surrounded by basement membrane components (arrow head). Leydig cell progenitors (Spindle-shaped cells located at the peritubular region and positive for 3ß-HSD) were absent in 1L hamster testis. Leydig stem cells/mesenchymal cells (M) are negative for 3ß-HSD.

Table 2. Testicular androgen secretory capacity *in vitro*, plasma T4, LC number and average LC volume (Size) in early pubertal 14L and 1L hamsters.

Parameter	14L Hamsters (14hr:10hr Light:Dark)	1L Hamsters (1hr:23hr Light:Dark)
LH-stimulated testosterone secretion *in vitro*/testis (ng)	2.05±0.05	2.98±0.03*
LH-stimulated androstenedione secretion *in vitro*/testis (ng)	3.00±0.32*	1.98±0.1
Plasma T4 (ng/ml)	54.9±2*	29.2±1.5
Leydig cell number/testis (10^6)	13±0.5*	4.63±0.2
Average volume of a Leydig cell (μm^3)	300±14	750±18*

Mean±SE. An asterisk (*) depicts a greater ($p<0.05$) value between the means of each parameter in 14L and 1L hamsters.

3ß-HSD label were identified as newly formed adult type LC (ALC, Fig. 1A, Ariyaratne and Mendis-Handagama, 2000). In 1L hamsters, clusters of large cells, which were surrounded by basal membrane components positive for 3ß-HSD, were observed and they were identified as fetal Leydig cells (FLC, Huntaniemi and Pelliniemi, 1992). Less frequently, these cells were observed as single cells in the testis interstitium. The identification of FLC was confirmed by regular light microscopy, with the characteristic features of abundance of large lipid droplets in their cytoplasm (not shown) in addition to the above mentioned characteristic features. 3ß-HSD positive cells similar to Leydig progenitors and newly formed ALC were not observed in testes of 1L hamsters.

Discussion

The present study examined the photoperiodic effects on the testis in golden hamsters in early puberty. Despite the fact that previous studies measuring testis size have not been able to detect photoperiodic differences in golden hamsters until seven weeks of age, present study demonstrated structural and functional changes within the testis at four weeks of age. To our knowledge, findings of the present study are the earliest reported photoperiodic differences in this species. These effects may be the initial changes in testes of short day hamsters that causes testicular atrophy after several weeks.

The present study showed that the Leydig stem cell differentiation in the postnatal hamster testis is arrested under reduced day lengths, even though testicular growth of both experimental groups has begun and show no difference in their testicular weights.

To our knowledge this is the first study that demonstrated a regulatory role of light on prepubertal Leydig stem cell differentiation in a seasonally breeding mammal, specifically the golden hamster. Whether this phenomenon is true for all mammals and other seasonal breeding mammals is an important issue to be addressed in future investigations.

To our knowledge, the information on Leydig cell parameters and hormonal (testosterone, androstenedion and T4) differences reported in the present study are the earliest photoperiodic differences reported for golden hamsters. Prior to the present study, the earliest known photoperiodic difference in this species was the circulating levels of prolactin, which occurs at 35 days of age (Donham et al., 1994).

Although we are aware that a lengthy non-stimulatory photoperiod of 1L hamsters is unlikely to be encountered during postnatal developing period of Golden hamsters, we used the same non-stimulatory photoperiod of 1:23 LD and the same stimulatory photoperiod of Darrow et al. (1980), which allowed us to compare our findings with the existing information in the literature on this subject. The choice of the photoperiods can also be justified by the report of Elliot (1976). Results obtained from the present investigation revealed new and interesting information and added new insight to the effects of photoperiod on Leydig stem cell differentiation in the postnatal testis. Moreover, findings were helpful in understanding the previously published data (Darrow et al., 1980) on the effect of photoperiod on reproductive development in the golden hamster.

1L hamsters were heavier than those raised under 14L conditions. Pitrosky et al. (2003) reported that reduced light conditions in Syrian/golden hamsters increase the accumulation of interscapular adipose tissue. In the present study, we observed abundant subcutaneous fat in 1L hamsters compared to 14L hamsters, possibly resulting from a similar fat depositing mechanism under short day lengths as reported by Pitrosky et al. (2003). This observation explains the increased body weight in 1L hamsters. Increased body mass has been observed in adult golden hamsters exposed to short day lengths; they increase their carcass lipid (Bartness and Wade, 1984, 1985) similar to the 1L hamsters of the present study. By contrast, Darrow et al. (1980) failed to observe a photoperiodic difference in body mass of golden hamsters during reproductive development. We cannot explain this difference.

It is important to note that spindle-shaped cells positive for 3ß-HSD, which were identified as Leydig progenitor cells, were exclusively seen in the peritubular region in testes of 14L hamsters. This observation supports the concept that the peritubular mesenchymal cells are the Leydig stem cells in the prepubertal hamster testis, similar to the prepubertal rat (Ariyaratne et al.,

2000a-c) and mouse testes (O'Shaughnessy et al., 2005). Moreover, absence of such cells in 1L hamsters suggests that Leydig stem cell differentiation in golden hamsters requires photostimulation (14:10 LD), and is inhibited under a non-photostimulatory condition of 1:23 LD.

14L hamsters had a greater capacity to secrete androstenedione. This observation is also supported by the results on the number of LC per testis, because androstenedion is one of the major androgens secreted by the newly formed adult leydig cells (Mendis-Handagama and Ariyaratne, 2001). The LC present in 1L hamsters were larger and reduced in number compared to 14L hamsters. They were mostly in clusters and were surrounded by basement membrane components. These features are characteristics of FLC (Huhtaniemi and Pelliniemi, 1992). Additionally, the higher testosterone secretory capacity of testes of 1L hamsters is in agreement with the presence of FLC in their testes, because they have a greater capacity than the adult LC to secrete testosterone (Huhtaniemi and Pelliniemi, 1992). These observations explain why the weights of testis and accessory sex glands were unchanged in 1L hamsters up to seven weeks of age (Darrow et al., 1980).

In prepubertal rats under transient neonatal thyroid hormone deficiency, fetal Leydig cells do not show regressive changes and therefore, testosterone secretory capacity of their testes do not decline (Mendis-Handagama and Ariyaratne, 2004) despite the absence of postnatal differentiation of new Leydig cells. However, with prolonged thyroid hormone deficiency, testicular testosterone secretory capacity of these rats declines as a result of the atrophy of the fetal Leydig cells and absence of postnatal differentiation of new Leydig cells (Mendis-Handagama and Ariyaratne, 2004). Based on this observation, it is possible to suggest that testosterone produced by the fetal Leydig cells in postnatal testes of 1L hamsters were capable of producing testicular growth, similar to the testicular growth in 14L hamsters, at least until 28 days of age. It is also been shown that regardless of the photoperiod before 15 days of age, testis weights of Djungarian hamsters increase similarly under short and long days but subsequently regress (Yellon and Goldman, 1984). Similarly, 12L:12D raised Turkish hamsters have shown a small amount of gonadal growth equivalent to 16L hamsters until five weeks of age (Hong and Stetson, 1986). These transient increases also could be evidence of fetal Leydig cell testosterone secretion.

When prepubertal golden hamsters were raised under 1hr:23hr LD, or blinded from birth to seven weeks of age, their circulating testosterone levels do not decline (Darrow et al., 1980). These observations agreed with the present findings and suggest that FLC in postnatal testes of 1L hamsters are capable of maintaining the circulating testosterone levels comparable to those of 14L. Based on these observations, these authors (Darrow et al., 1980) concluded that the onset of puberty in the golden hamster is not photoperiodically controlled. However, they also reported (Darrow et al., 1980) that continuation of the blind state up to 13 weeks, resulted in atrophy of testis and seminal vesicles (testosterone dependent). Moreover, other studies report similar findings. According to Sisk and Turek (1987), the reproductive system of the male golden hamster is refractory to the inhibitory effects of short days until five to six weeks of age and thereafter, exposure to short day lengths initiates testicular regression. Similarly, Rollag et al. (1982) demonstrated that testis weights in golden hamsters decrease at ninth and tenth week of postnatal life following blinding or melatonin injections.

Based on the findings of the present investigation and our previous observations (Mendis-Handagama and Ariyaratne, 2004), we suggest that the above results are suggestive of atrophy of fetal Leydig cells at that age together with the absence of postnatally differentiated Leydig cells in 1L hamsters and cause testicular regression.

It is reported that testosterone levels increase during puberty in golden hamsters (Zehr et al., 2006). It is also interesting to note that the effect of short days on testicular testosterone secretory capacity is different between 28 day old hamsters and adult hamsters. The present study showed that testicular testosterone secretory capacity in 1L hamsters is not lowered by exposure to 28 days of short day lengths. By contrast, the adult male hamsters subjected to short days had significantly lower circulating testosterone levels than those subjected to long days (Frungieri et al., 2005). These differences could be explained by the populations of LC that are present in their testicles. Leydig cells in adult hamsters, which were postnatally differentiated, regress and secrete low levels of testosterone when exposed to short days (Bartke et al., 1996). However, it appears that FLC present in testes of 1L hamsters secrete an abundance of testosterone up to seven weeks of age (Darrow et al., 1980), although postnatal LC differentiation is arrested in their testes; unchanged accessory sex gland weights observed in 1L hamsters in the study of Darrow et al. (1980) are in agreement with this finding. However, Sisk and Turek (1983) did not detect photoperiodic differences in circulating testosterone concentrations through five weeks of age; it is not possible to explain this discrepancy.

During development, prolactin levels in golden hamsters increase between 25-30 days (Donham et al., 1994), a similar age as 1L hamsters of the present study. It is also reported that hamsters exposed to short day lengths (i.e. 5h light per day) exhibited significantly less LH binding in testes than those exposed to 14h light per day. Latter finding agrees with the observation of reduced number of Leydig cells per testis in 1L hamsters of the present study. Additionally, such findings question whether low numbers of Leydig cells in hamsters exposed to short day lengths are due to an arrest in Leydig cell differentiation caused by an inhibitory effect of longer duration of circulating prolactin levels in such hamsters.

Under short day lengths, the duration of melatonin secretion by the pineal gland is increased (Vriend, 1985; Skene and Arendt, 2006). Therefore, it is logical to accept that virtually all photoperiodic changes observed in hamsters are due to the exposure to short days; these changes include the inhibition of thyroid hormone secretion by the thyroid gland (Vriend, 1985; Vriend and Thliveris, 1985; Saita et al., 2005; Revel et al., 2006). Based on the fact that thyroid hormones are crucial for the process of postnatal Leydig cell differentiation (Mendis-Handagama et al., 1998; Ariyaratne et al., 2000a-c), the reduced number of Leydig cells per testis in 1L hamsters of the present study could also be attributed to the arrest in postnatal LC differentiation as a result of low levels of circulating T4 in 1L hamsters. This suggestion is also supported by the observations of Jansen et al (2007); they reported that in golden hamster, full complement of adult reproductive functions requires thyroid hormones during early postnatal period.

In contrast to the present findings on low circulating levels of plasma T4 in 1L hamsters, Donham et al. (1994) failed to observe a photoperiodic effect on T4 concentrations in Syrian hamsters at 21, 36 or 48 days of age. The reasons for this discrepancy are not clear at present.

In conclusion, findings of the present study demonstrated that despite the unchanged testis weight/size, structural and functional changes occur in testes of early pubertal golden hamsters exposed to short days; postnatal Leydig cell differentiation in the golden hamster is arrested with short photoperiodic exposure from birth to 28 days of age. Additionally, findings of the present study is in agreement with the conclusion that puberty in golden hamsters is photoperiodically controlled.

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