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The effects of photoperiod and melatonin injections on the reproductive system of the male Mongolian gerbil (Meriones unguiculatus)

Luis Jose Cova Marchan

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THE EFFECTS OF PHOTOPERIOD AND MELATONIN INJECTIONS ON THE REPRODUCTIVE SYSTEM OF THE MALE MONGOLIAN GERBIL (MERIONES UNGUICULATUS)

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

OF THE UNIVERSITY OF RICHMOND

IN CANDIDACY

FOR THE DEGREE OF

MASTER OF SCIENCE IN BIOLOGY

MAY, 1934

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LUIS JOSE COVA MARCHAN

THE EFFECTS OF PHOTOPERIOD AND MELATONIN INJECTIONS ON THE REPRODUCTIVE SYSTEM OF THE MALE MONGOLIAN

GERBIL (MERIONES UNGUICULATUS)

BY

LUIS JOSE COVA MARCHAN

APPROVED:

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ABSTRACT

This work was undertaken to study the effects of photoperiod and melatonin on fertility in male Mongolian gerbils (Meriones unguiculatus). Four groups of fifteen animals each, were placed in different photoperiods (ambient, ranging from 14L:lOD to ll.3L:l2.3D; 24L:l0D; 14L:l0D; and 1L:23D). After 35 days, three gerbils from each photoperiod condition were sacrificed and the testes, epididymides, seminal vesicles, prostate, kidneys, adrenals, thymus and hypophysis were weighed. The testes and epididymides were placed in Bouin's fixative and used for histological studies. Also, two males from each photoperiod condition were paired individually with a female. The remaining animals of each photoperiod condition were divided into two subgroups that received melatonin or vehicle injections for 35 days. After that, the gerbils were killed and the same organs examined.

Photoperiod alone or combined with melatonin injection neither enhanced nor inhibited the body weight, sexual organs and other organs by 70 days. The presence of spermatozoa in both the testes and epididymides, and the offspring obtained of the animals exposed to different photoperiods and melatonin, indicate no dependence of the process of sexual maturation and reproduction in the Mongolian gerbil on these factors.

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INTRODUCTION

It is well documented that light acts as an important environmental factor in the regulation of reproductive cycles of vertebrates. Experiments with birds as well as with mammals, reptiles and fishes have demonstrated the effects of photoperiod on gonadal recrudescence (Axelrod, 1974). Studies of photoperiod in mammals have been principally with rats (Wurtman et al., 1963; Reiter et al., 1966) and hamsters (Reiter et al., 1974; Turex, 1979). Female rodents exposed to shortened photoperiods $($ < 10 hr. light) and to complete darkness had reduced ovarian and uterine wt., a decreased incidence of estrus and frequency of ovulation and reduced numbers of offspring (Wurtman et al., 1963; McIssaac et al., 1964; Reiter et al., 1966; Reiter et al., 1975b). While the effects of photoperiod on female mammals are clear, effects on males are controversial. Exposure to daily photoperiods greater than 12 hr., resulted in an enlargement of the testes and secondary organs in rats and hamsters (Hoffman and Reiter, 1965a; Reiter and Sorrentino, 1970; Reiter et al., 1970; Turek, 1979; Stetson and Tate-Ostroff, 1981), whereas exposure to a constant darkness gives an opposite effect (Reiter, 1968; Reiter et al., 1974; Alonso et al., 1978;
Moss et al., 1979). Although these studies indicate males are similar to females, many studies on males are not as clear with animals showing no response to photoperiod (Reiter et al., 1974; Turek et al., 1975; Shugart, 1980; Constantine, 1981).

The pineal gland has been implicated in regulating photoperiodic influence in photosensitive animals (Reiter, 1973a). In mammals, the pineal originates in the brain of the embryo and loses all nerve connections with the brain soon after birth (Axelrod, 1974). It is derived phylogenetically from photoreceptor cells but has lost its ability to generate impulses in response to environmental light (Uurtman and Axelrod, 1965; Axelrod, 1974). Its primary action is as a neurotransducer, an element that converts incoming nervous impulses originated by changes of light periods in the environment into chemical messages that act on the secretion of gonadotrophins of the hypophysis (Wurtman and Axelrod, 1965; Collu and Fraschini, 1972). Nerve impulses are carried to the pineal by fibers of the sympathetic nervous system which release norepinephrine (Wurtman and Axelrod, 1965). Stimulation of the nerve fibers by light inhibits the synthesis and release of melatonin by the pineal (Wurtman and Axelrod, 1965; Axelrod, 1974). This compound and serotonin have been implicated in the photoperiodic involvement of the pineal with gonadal recrudescence. Melatonin acts on the gonads because of its influence on the hypothalamicpituitary region, where it causes a decrease in the output of FSH and LH (Walker et al., 1982; Probst and Feske, 1982).

Studies conducted on relations of the pineal to photoperiod and reproduction in female mamnals (e.g., rats and hamsters) have been numerous (Wurtman et al., 1963; Chu et al., 1964; Wurtman and Axelrod, 1965, and Kinson and Robinson, 1970),

whereas those conducted on this relation in males are sparse and results variable. Exposure of male hamsters, rats, mice and gerbils to longer photoperiods induces the enlargement of the testes and accessory sexual organs whereas exposure to short daily light or blindness causes atrophy of the reproductive system (Reiter, 1968; Reiter and Fraschini, 1969; Reiter et al., 1970; Reiter at al., 1974; Turek et al., 1975; Moos et al., 1979). Results of many studies on the effects of pinealectomy of male mammals, however, are inconclusive. It induced enlargement of testes and accessory sexual organs in hamsters (Reiter et al., 1974) and rats (Collu and Fraschini, 1972; Vaughan and Reiter, 1973; however in a later study (Reiter et al., 1975a) no effects were found in rats.

Administration of melatonin to male rats, hamsters, mice and gerbils has given ambiguous results. In some instances melatonin caused a reduction in the weight of both the ventral prostate and the seminal vesicles (Debeljuk, 1969; Kinson and Peat, 1971) but in others there were no effects on the testes and accessory sexual organs (Kinson and Robinson, 1970; Hoffman, 1973; Turek et al., 1975; Shugart, 1980; Constantine, 1981).

Meriones unguiculatus, the Mongolian gerbil a desert mammal was selected for this study as it has not been inbred for as long as most laboratory animals and therefore might prove more susceptible to changes in photoperiod. During good weather the gerbil is active either day or night (Robinson, 1959; Walker, 1964) but in winter weather it spends most of its time in

burrows (Moos et al., 1979). In the present study gerbils were subjected to a combination of photoperiod and melatonin injections in an effort to determine their effects on the development of the gonads, accessory sexual organs and on fertility.

MATERIALS AND METHODS

Sixty-three (sexually inmature) 8-9 week old male Heriones unguiculatus were obtained from Tumblebrook Farms, West Brookfield, MA. in August, 1983. Animals at this age were chosen in order to study the effects of photoperiod and melatonin on sexual development. Meriones unguiculatus achieves sexual maturity in approximately 12 weeks (Marston and Chang, 1965). The gerbils were housed in plastic and wire-mesh cages during a one-week acclimation. They were kept under a 14L:l0D photoperiod in a secluded room with ambient temperature of 24°-26° and given water and Purina lab chow ad libitun. The animals were weighed on arrival and then periodically throughout the experiment.

Three males were randomly selected and killed by cervical dislocation. Testes, epididymides, seminal vesicles, prostate, kidneys, adrenals, pituitary and thymus were removed and weighed. A mean weight of the paired organs (e.g., testes) was recorded. The testes and epididymides were placed in Bouin's fixative and used for histological examination. This group is designated Initial Control. The remaining 60 males were randomly divided into four groups of 15 animals each and used for the photoperiod assay. Group one was housed in a cage 1.0 X 0.5 X 0.5 $\text{\texttt{m}}$ provided with secluded areas to hide the animals. Then cages were maintained under natural day-light conditions in a greenhouse with photoperiods ranging from 14L:lOL to ll.3L:l2.3D (tenperatures 18°-21°). Animals of the other three groups were housed

individually in plastic cages, measuring 28.8 X 17.5 X 13.0 cm with wire tops. Group two gerbils were placed in a Keysor-Scheer model CEI-36-10 environmental chamber and exposed to a photoperiod of 14L:l0D with lights on from 2300 hr. to 1300 hr. Animals in group three were housed in a Keysor-Scheer model CEL-36-10 environmental chamber and exposed to a photoperiod of 1L:23D (lights on from 1300 hr. to 1400 hr.). Group four gerbils were placed in a Biotronette Mark III environmental chamber (Lab-line Instruments Inc.) in a 24L:OD photoperiod with lights on continuously. Six 40w fluorescent bulbs that produced an intensity of 45-55 lumens per square foot were used in the three environmental chambers. Temperatures in the chambers were between 23°-27° during the entire experiment. All animals were fed with Purina lab chow and tap water ad libitum.

After 35 days, three gerbils from each photoperiod were killed, weighed and necropsied. Two animals from each photoperiod were paired with females. The remaining 10 animals were maintained in the original conditions of photoperiod for an additional 35 days. They were sub-divided into two groups and each received daily subcutaneous injections. One subgroup received injections of 10 pg melatonin prepared in 50 pl of 3% ethanol in Locke's physiological solution for marmals (ELP)(Zarrow et al., 1964). The second subgroup received vehicle (ELP) injections. After 35 days, two males from each photoperiod and injection regimen were placed in individual cages and paired with a female. The remaining males were killed and necropsied.

Fertility Study

Twenty-four, 20-24 week old female Mongolian gerbils were obtained from Tumblebrook Farms, MA. and used for mating. They were maintained under a 14L:l0D photoperiod (lights on from 0800 hr. to 2200 hr . and mated with males from the various photoperiod regimens. On day 20 the males were removed. The number of offspring was counted at birth. As the gestation period of Mongolian gerbils is 24-26 days (Harston and Chang, 1965; Norris and Adams, 1981), only the young born within 30 days of the initial mating were counted.

Histological Procedures

After weighing, testes and epididymides from each animal were placed in Bouin's fixative for at least two days, dehydrated with a series of alcohols (35% to 100%) and then placed in xylene. The tissues were embedded in Paraplast and sliced at 8 µm using a rotatory microtone. Sections were attached to microscope slides with egg albumin, rehydrated, and stained for 30 min. with Delafield's hematoxylin. Lithium carbonate solution was used to blue the stain. The slides were dehydrated and mounted in Pernount and examined under microscope for sperm and general development.

Statistical Analysis

Analysis of variance (ANOVA) was perforned using the Statistical Package for the Social Sciences (SPSS, Northwestern University). A one way ANOVA was perforned on the body weight and on all organ weights, expressed as percentages of total body weight, on animals necropsied after 35 days. Duncan's multiple range test was used to order means and group means into similar groups. A two way ANOVA was executed on all organ weights. The Student's t-test was used to test differences (0.05 confidence level).

RESULTS

The testes of the gerbils used as initial controls showed minimal development of the seminiferous tubules and reduced spermatogenesis was observed (Fig. 1). Also, the epididymides in these animals were underdeveloped and transverse sections had few spermatozoa (Fig. 1) denoting incomplete sexual maturity of the animals at the beginning of the experiment.

At the end of 35 days the body weight of the animals kept in the Greenhouse was significantly larger than that of the other groups. Also the weight of the animals at 1L:23D was significantly higher than the other groups at this time. All body weights differences (even in groups injected with melatonin) disappeared by 70 days (Table 1).

By 35 days, the animals kept in the 14L: lOD photoperiod had significantly larger testes (Fig. 2), epididymides (Fig. 3) and seminal vesicles (Fig. 4) when compared to the other groups. No variation was observed in prostate weights (Fig. 5). Also, the 24L:OD group had a significantly larger testis and seminal vesicle weights than the other two groups. The testes of the 1L:23D group was larger than the Greenhouse group but the seminal vesicles of the Greenhouse group were larger than the 1L:23D group. Histological analysis of the testis sections indicated no appreciable variation among the animals at 35 days. In the majority of the seminiferous tubules, spermatids and mature spermatozoa were observed (Fig. 6). Similarly, the epididymis sections showed an accumulation of spermatozoa in

all the animals of the different photoperiods (Fig. 7) indicating the sexual maturity of the gerbils.

Kidney and thymus weights of the animals kept in the 1L:23D photoperiod were significantly larger when compared to the other groups at 35 days. Also, kidney weights were significantly larger in the 14L:l0D group than in the 24L:OD and Greenhouse groups. Conversely, thymus weights were larger in gerbils of the 24L:OD and Greenhouse groups than in the animals of 14L:l0D group (Table 2).

By 70 days, the mean weights of the testes (Fig. 8), epididymides (Fig. 9), prostate (Fig. 10) and seminal vesicles (Fig. 11) did not differ. Histological analysis of the testis sections showed spermatogenetic activity (Fig. 12 and Fig. 13) and the epididymis sections of all the animals kept in different photoperiods had an accumulation of spermatozoa (Fig. 14 and Fig. 15).

No significant variation was observed in the mean weights of kidneys, adrenals, thymus and pituitary among the animals kept until 70 days (Table 3). These data indicate that neither photoperiod nor melatonin influenced the weight of these organs.

The numbers of offspring from fenales bred by the males from the different photoperiods at 35 days are shown in Fig. 16. The females mated to the 2 males from the 24L:OD were both pregnant producing 6 offspring each. Only one female each from the Greenhouse and 1L:23D groups had offspring (6 each). No pregnancies were obtained from the females mated with males from

the 14L:l0D photoperiod. The progeny of males from 70 days groups that received vehicle and melatonin injections are shown in Fig. 17. As can be seen, females mated to males from all photoperiodic and melatonin regimens produced 4-8 offspring.

Contrary to expectation, the weights of the primary and secondary sexual organs as well as the other organs were not significantly larger by 70 days when compared to initial controls. Only prostates and adrenals were significantly larger than the initial controls at 35 days (Table 4).

DISCUSSION

While there was a small increase in body weight in the animals exposed to the short photoperiod in the initial 35 days of the study, this difference soon disappeared and at the conclusion of the experiment there were no differences in body weight of the animals in the various groups. This finding agrees with studies in golden hamsters, Mesocricetus auratus, (Heldmaier and Hoffman, 1974; Bartness and Wade, 1934) and white rats (Reiter and Fraschini, 1969), and it is concluded that the normal growing phase of the Mongolian gerbil is not influenced by photoperiod and melatonin.

Testicular weights in the Mongolian gerbil were unaffected by photoperiod. This does not conform to studies of Moos et al. (1979) and Clark and Galef (1981) who found an inhibitory effect of short photoperiod on the testes of gerbils. Similar inhibitory effects have been found in white rats (Reiter et al., 1968: Kinson and Peat, 1971) and hamsters (Hoffman and Reiter, 1965a; Hoffman and Reiter, 1965b).

Melatonin apparently had no effect on testicular development in animals used in the present experiment. Comparable results have been observed in white rats (Kinson and Robinson, 1970; Reiter et al., 1975a; Turek $et al.$, 1975; Alonso $et al.$,</u></u> 1978; Lang et al., 1983), mice (Shugart, 1980; Constantine, 1981) and hamsters (Hoffman, 1973; Reiter et al., 1975a; Reiter et al., 1976). However, not all studies are in agreement as several researchers have found that melatonin exhibited an inhibitory

effect on testicular weights in white rats (Kitay and Altschule, 1954; Reiter, 1968; Debeljuk, 1969; Debeljuk et al., 1971; Turek, 1977; Reiter et al., 1978) and hamsters (Reiter, 1973b; Reiter et al., 1975a; Tamarkin et al., 1977b; Reiter et al., 1978). Histological studies support the testicular growth data in the present experiment. Spermatozoa and open seminiferous tubules were observed in all sections. The spermatogenesis process observed in the testes of the animals placed in diverse photoperiod and melatonin injections was not different. This finding agrees with other works done with melatonin injected mice (Shugart, 1980), pinealectomized mice (Constantine, 1981) and pinealectomized white rats (Baum, 1968). Reiter and Sorrentino (1970) and Hoffman (1973) however show involution of the testes of the hamsters that were kept in low photoperiods.

Melatonin probably acts through the hypothalamus-hypophysis axis inhibiting the secretion of gonadotrophins (Reiter et al., 1968; Debeljuk, 1969; Hoffman, 1973; Reiter et al., 1975b; Turek et al., 1975; Tamarkin et al., 1976; Alonso et al., 1978; Walker et al., 1982) or directly inhibits the gonads (Chu et al., 1964; Debeljuk et al., 1971). Curiously, melatonin seems to act as a progonadal agent stimulating the secretion of gonadotrophin hormones in female white rats (Fiske et al., 1984), female hamsters (Reiter et al., 1965b) and male hamsters (Reiter et al., 1975a). When chronic amounts of circulating melatonin are present, a refractory state develops. Under these conditions melatonin does not inhibit the reproductive development (Reiter et a_{1} , 1968).

The only photoperiod to have a significant effect on the development of the epididymis in the present study was the ·~ intermediate photoperiod (14L:lOD) which was stimulatory at 35 days. White rats (Reiter, 1968), mice (Shugart, 1980) and hamsters (Reiter and Hester, 1966; Reiter, 1968; Reiter et al., 1974) responded differently in that there was an inhibitory effect on the epididymis during the short photoperiod. The presence of mature spermatozoa in the epididymis sections in all groups indicate that neither photoperiod nor photoperiod combined with melatonin affected the maturation of the epididymis.

Prostate weights of gerbils were not influenced by photoperiod or melatonin. However, longer photoperiods (e.g., 241: OD) were stimulatory to the weight of seminal vesicles measured at 35 days. These two structures have been correlated with sexual maturity in mammals and their developments are influenced by the secretion of LH. Several researchers have demonstrated that short photoperiod and/or melatonin inhibits development of these organs in white rats (Wurtman et al., 1964; Reiter et al., 1964; Reiter et al., 1968; Kunkel, 1969; Debeljuk, 1969; Kinson and Robinson, 1970; Sorrentino et al., 1971; Debeljuk et al., 1971; Kinson and Peat, 1971; Alonso et al., 1978), mice (Vaughan and Reiter, 1971; Shugart, 1980; Constantine, 1981) and hamsters (Hoffman and Reiter, 1965b; Reiter and Hester, 1966; Reiter et al., 1966; Reiter et al., 1974; Reiter et al., 1975a; Turek, 1979; Stetson and Tate-Ostroff, 1981). One study (Kinson and Robinson, 1970) found that light restriction did not alter the weight of the prostate and seminal vesicle of white rats.

There is considerable controversy concerning the effect of melatonin and photoperiod on the weights of the pituitary, kidneys, adrenals, and thymus of both male or female rodents. Hoffman and Reiter (1965b), Gromova et al. (1967) and Reiter et al. (1975a) had positive results. Similar studies by Reiter et al. (1966), Sorrentino et al. (1971) and Shugart (1980) indicate no such influence. It is clearly demonstrated in the present study that these organs in Mongolian gerbils are unaffected by photoperiod and melatonin in long term exposure (70 days).

The pregnancies and the offspring obtained indicate the sexual maturity in all groups exposed to photoperiod and melatonin injections. The fact that some females did not become pregnant could be due to female infertility.

In conclusion, there was no evidence to support the hypothesis that the development of primary and secondary sexual organs or any other organs associated with reproduction and fertility are dependent on photoperiod or melatonin in Meriones unguiculatus.

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Table 1. Body weights of male Mongolian gerbils (g), (Mean \pm SD), N = 3.

 $\Delta \sim 10^{11}$ m $^{-1}$

A) Initial Control: 62.60 ± 15.02

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** $P \le 0.01$

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*** $P \subset 0.001$

Table 2. Weights of different organs of male Mongolian gerbils after 35 days exposure to different photoperiods (Mean \pm SD). Values are given in % of total body weight, $N = 3.$

	PHOTOPERIOD					
	Greenhouse	24L:OD	14L:10D	1L:23D		
Kidney	4.28 \pm 0.05	4.32 \pm 0.18	4.84 \pm 0.25 ^{**}	$5.30 \pm 0.11***$		
Adrenal	0.38 ± 0.03	0.43 ± 0.02	0.38 ± 0.13	0.48 ± 0.08		
Thymus	1.20 ± 0.09	1.41 ± 0.12 **	1.14 ± 0.12	1.66 ± 0.05 **		
Hypophysis	0.03 ± 0.002	0.06 ± 0.005	0.06 ± 0.027	0.04 ± 0.004		

** $P < 0.01$

 $***$ P < 0.001

 \mathbf{x}

Table 3. Weights of different organs of male Mongolian gerbils after 70 days exposure to different photoperiod and 35 days of injection (Mean \pm SD). Values are given in % of total body weight, $N = 3$.

	PHOTOPERIOD				
	Greenhouse	24L:OD	14L:10D	1L:23D	
Kidney					
vehicle	3.89 \pm 0.14	4.61 \pm 0.04	4.88 \pm 0.04	4.93 ± 0.30	
melatonin	3.57 ± 0.14	4.27 ± 0.27	4.83 ± 0.36	5.30 \pm 0.48	
Adrenal					
vehicle	0.32 ± 0.06	0.40 ± 0.02	0.45 ± 0.13	0.33 ± 0.04	
melatonin	0.32 ± 0.07	0.44 ± 0.03	0.36 ± 0.03	0.43 ± 0.09	
Thymus					
vehicle	0.75 ± 0.16	1.06 ± 0.11	0.72 ± 0.06	0.96 ± 0.06	
melatonin	0.81 ± 0.12	0.90 ± 0.12	0.64 ± 0.10	0.98 ± 0.11	
Hypophysis					
vehicle	0.03 ± 0.03	0.05 ± 0.00	0.05 ± 0.01	0.04 ± 0.03	
melatonin	0.06 ± 0.04	0.04 ± 0.01	0.03 ± 0.02	0.06 ± 0.03	

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Table 4. A comparison of organ weights of Initial Control gerbils with Greenhouse maintained control gerbils at 35 days and 70 days, (Mean \pm SD), N = 3. A = Initial Controls; $B =$ Greenhouse at 35 days; $C =$ Greenhouse at 70 days.

\mathbf{A}	B	$\mathbf C$	
6.19 ± 1.42	4.40 ± 1.11	7.49 ± 0.68	
0.91 ± 0.33	0.81 ± 0.33	1.55 ± 0.11	
0.06 ± 0.07	0.54 ± 0.15	0.68 ± 0.20	
0.65 ± 0.20	1.95 ± 0.15	1.40 ± 0.21	
3.33 ± 0.59	4.28 \pm 0.05	3.89 ± 0.14	
0.23 ± 0.01	0.38 ± 0.03 **	0.32 ± 0.06	
2.96 ± 0.77	1.20 ± 0.09	0.75 ± 0.16	
0.04 ± 0.01	0.03 ± 0.02	0.03 ± 0.03	

 $*$ P<0.05

** $P < 0.01$

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Fig. 1. Photomicrographs of testis and epididymis sections of Mongolian gerbils used as initial controls. Magnification is 250X.

 $A = Testis$

B = Epididymis

Fig. 2. The effects of photoperiod on testis weight of the Mongolian gerbil at 35 days (Mean \pm SD), N = 3.

Fig. 3. The effects of photoperiod on epididymis weight in the Mongolian gerbil at 35 days (Mean \pm SD), N = 3.

Fig. 4. The effect of photoperiod on seminal vesicle weight of the Mongolian gerbil at 35 days (Mean \pm SD), N = 3.

Fig. 5. The effect of photoperiod on prostate weights of the Mongolian gerbil at 35 days (Mean \pm SD), N = 3.

Fig. 6. Photomicrographs of testis sections of Mongolian gerbils exposed to different photoperiods for 35 days. Magnification is 2SOX.

- 1. Greenhouse
- $2. 24L:OD$
- 3. 14L:10D
- 4. 1L:23D

- Fig. 7. Photomicrograpbs of epididymis sections of Mongolian gerbils exposed to different photoperiods for 35 days. Magnification is 250X.
	- 1. Greenhouse
	- $2. 24L:OD$
	- 3. 14L:l0D
	- 4. 1L:23D

The effect of photoperiod and melatonin on testis Fig. 8. weight of the Mongolian gerbil at 70 days (Mean \pm SD), N = 3.

Greenhouse 24 L: 1 D

 $14L$: $10D$ $1L$: $23D$

Fig. 9. The effects of photoperiod and melatonin on epididymis weight of the Mongolian gerbil at 70 days (Mean \pm SD), N = 3.

 $14L:10D$ $1L: 23D$ Greenhouse 24L: ID

Fig. 10. The effects of photoperiod and melatonin on prostate weights of the Mongolian gerbil at 70 days (Mean \pm SD), N = 3.

vehicle

Fig. 11. The effects of photoperiod and melatonin on seminal vesicle weight of Mongolian gerbils at 70 days (Mean \pm SD), N = 3.

vehicle

Fig. 12. Photomicrographs of testis sections of Mongolian gerbils exposed to different photoperiods for 70 days and melatonin injections for the last 35 days. Magnification is 250X.

- 1. Greenhouse
- $2. 24L:OD$
- 3. 14L:l0D
- 4. 1L:23D

- Fig. 13. Photomicrographs of testis sections of Mongolian gerbils exposed to different photoperiod for 70 days and vehicle injections for the last 35 days. Magnification is 250X.
	- 1. Greenhouse
	- $2. 24L:OD$
	- 3. 14L:l0D
	- 4. 1L:23D

Fig. 14. Photomicrographs of epididymis of sections of Mongolian gerbils exposed to different photoperiods for 70 days and melatonin injections for the last 35 days. Magnification is 250X.

- 1. Greenhouse
- $2. 24L:OD$
- 3. 141:100
- $4. 1L:23D$

Fig. 15. Photomicrographs of epididymis sections of Mongolian gerbils exposed to different photoperiods for 70 days and vehicle injections for the last 35 days. Magnification is 250X.

> 1. - Greenhouse $2. - 24L:OD$ 3. - 14L:l0D 4. - 1L:23D

Fig. 16. Progeny of male gerbils subjected to various photoperiods for 35 days after a single mating.

Fig. 17. Progeny of male gerbils subjected to various photoperiods for 70 days and melatonin and vehicle injections for 35 days after a single mating.

vehicle injections

melatonin injections

VITA

Luis Jose Cova Marchan was born Harch 11, 1947 in Cumana, Venezuela. He received his elementary education at "Rendon" school and secondary education at Liceo "Antonio Jose de Sucre," Cumana. He got the Bachiller in Science degree in 1965. He attended the University of Oriente, Cumana, and received a degree of Licenciate in Biology in September, 1971. He was appointed Instructor of Biology at the University of Oriente, Bolivar, in March, 1972 and received a fellowship for graduate studies in 1981. He then attended graduate school at the University of Richmond, Virginia, U.S.A. and received a Master of Science degree in May, 1984.

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