

Short Day Lengths Delay Reproductive Aging¹

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ABSTRACT

Caloric restriction and hormone treatment delay reproductive senescence in female mammals, but a natural model of decelerated reproductive aging does not presently exist. In addition to describing such a model, this study shows that an abiotic signal (photoperiod) can induce physiological changes that slow senescence. Relative to animals born in April, rodents born in September delay their first reproductive effort by up to 7 mo, at which age reduced fertility is expected. We tested the hypothesis that the shorter day lengths experienced by late-born Siberian hamsters ameliorate the reproductive decline associated with advancing age. Short-day females (10L:14D) achieved puberty at a much later age than long-day animals (14L:10D) and had twice as many ovarian primordial follicles. At 10 mo of age, 86% of females previously maintained in short day lengths produced litters, compared with 58% of their long day counterparts. Changes in pineal gland production of melatonin appear to mediate the effects of day length on reproductive aging; only 30% of pinealectomized females housed in short days produced litters. Exposure to short days induces substantial decreases in voluntary food intake and body mass, reduced ovarian estradiol secretion, and enhanced production of melatonin. One or more of these changes may account for the protective effect of short day lengths on female reproduction. In delaying reproductive senescence, the decrease in day length after the summer solstice is of presumed adaptive significance for offspring born late in the breeding season that first breed at an advanced chronological age.

aging, female reproductive tract, melatonin, ovary, pineal

INTRODUCTION

Research on reproductive aging in mammals has focused mainly on rats and mice; within this restricted framework only very few of the available genotypes have been deployed [1, 2]. Much can be gained by extending the analysis to other models for human aging. Species with a range of aging rates can provide unique opportunities and novel insights [1] and increase confidence regarding generalizations between species and genotypes [2].

In the northern temperate zone, rodents with extended breeding seasons typically produce offspring from April through early October [3, 4]. A female's age at first reproductive effort is determined by the timing of her own birth.

Females that are born during the spring experience long and increasing day lengths (DLs), achieve sexual maturity at about 5 wk of age, and mate shortly thereafter. By contrast, young born after the summer solstice are exposed to decreasing DLs and delay reproductive maturation until the following spring, at which time they are 7 or more months old [reviewed in 5–7]. Few adults of the preceding year survive long enough to breed in the spring [5, 8]. This poses a reproductive conundrum: the breeding cohort in the spring consists primarily of over-wintering females that first mate at an age when fertility and fecundity would be expected to decline. For example, among Djungarian hamsters (*Phodopus campbelli*) maintained in a long day (LD) photoperiod, 80% of young females 3–5 mo of age littered successfully, but only 37% of females 8–10 mo old did so [9]. On these bases we predicted that middle-aged females (9 mo old) exposed from birth to short DLs would be less subject to reproductive senescence than age-matched females raised in long DLs.

To evaluate this conjecture we chose the Siberian hamster (*Phodopus sungorus*), which produces pups in the field from April to September [10]. Variations in DL transduced by the pineal gland determine the timing of sexual maturity. Females housed from birth in long DLs show early onset of estrous cycles, whereas puberty is delayed for several months in hamsters kept from birth in short DLs [11, 12]. Based on results from the closely related Djungarian hamster [9] and the marked reduction in reproductive success of older female Siberian hamsters [13], we expected that LD females would show substantial decreases in fecundity by 9 mo of age. Our principal goal was to determine whether age-matched, SD females would be protected from this projected decline in litter production. Because the effects of DL on the neuroendocrine axis are mediated by pineal melatonin secretion, we also evaluated the reproductive performance of pinealectomized animals kept in short days (SD). In this and other species, SD pinealectomized rodents are phenotypically similar to intact LD animals [14–17].

MATERIALS AND METHODS

Animals

Breeding pairs from our Siberian hamster colony were housed in either an LD (14L; 14L:10D, light onset at 0400 h) or transferred to an SD photoperiod (10L; 10L:14D, light onset at 0800 h) on the day of pairing. Their female offspring were housed in the natal DL from the time of conception through 36 wk of age. Food (Purina rodent chow 5015; Purina Mills, St. Louis, MO) and water were available ad libitum. During gestation and lactation, diets were supplemented with sunflower seeds. Offspring from some SD pairs were pinealectomized (PX) between Postnatal Days (PD) 2–4. Unexpected maternal cannibalism of PX pups by SD dams resulted in reduced sample size for the SD-PX group. Litters were weaned on PD18, and females were housed in groups of two or three per polypropylene cage (25 × 14 × 12 cm). SD and SD-PX females remained in the 10L photoperiod until 36 wk of age, at which time they were transferred to 14L for 4 wk. LD females remained in their original photoperiod throughout the study. Females were weighed weekly, and coat color and

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TABLE 1. Number and percentage of mated and pregnant females after initial pairing with a proven breeder male at 9 mo of age. Total number of failed pregnancies and etiology of failures for long day (LD), short day (SD), and short day–pinealectomized (SD-PX) females.

	LD	SD	SD-PX
No. paired	27	23	10
No. mating	26 (96%)	22 (96%)	10 (100%)
No. pregnant (% of mated)	22 (85%)	19 (86%)	7 (70%)
No. failed pregnancies	7	0	4
Maternal deaths	2	0	1
Complete stillborn litters	3	0	2
Complete losses in utero	2	0	1

vaginal patency were recorded. At 40 wk of age, each virgin female was transferred to a clean cage and paired with a proven breeder male for 10 days. For the first 3 days after pairing the compatibility of pairs was monitored. One SD female was separated from her mate and was excluded from the study because she sustained injuries. Each day females were inspected for the presence of a copulatory plug. Females were housed singly after removal of the male and inspected daily for presence of litters. On the day of parturition, numbers of live and dead pups were counted and litter mass of live pups was recorded (± 0.1 g). Females in which all pups were stillborn, or that failed to give birth 20 days after separation from the male, were killed by CO₂ inhalation. Their uteri were inspected for fetoplacental tissue and/or implantation scars. Litters were weaned on PD18 and pups individually weighed and sexed. Dams that weaned litters were administered a lethal dosage (i.p.) of sodium pentobarbital at 12 mo of age, and the number of uterine implantation scars was counted.

Histology

We collected reproductive tissues for histological examination at 13 and 26 wk of age in the expectation that SD females would differ significantly from LD females at the earlier but not the later age. By 26 wk of age we anticipated SD females would be refractory to the inhibitory effects of SD and sexually mature. At 13 wk of age, six LD and six SD females, selected at random from the larger groups, were killed by transcardial perfusion with 3% paraformaldehyde (PFA) under deep anesthesia induced with i.p. injection of sodium pentobarbital. Too few SD-PX females were available to contribute to this part of the study. An additional five animals from the LD and SD groups were killed at 26 wk of age. Uteri and ovaries were removed and dissected free of surrounding fat and weighed after overnight fixation in 3% PFA. Tissues were processed to obtain polyethylene glycol sections of 6- μ m thickness. Every 12th section of a single ovary, selected at random from the right or left side, was stained with hematoxylin and eosin, examined at 400 \times magnification, and the number of primordial follicles counted. Slides were randomized and examined by a single investigator who was unaware of the donor's treatment group. Follicle counts per ovary were estimated by multiplying by 12. This approach may overestimate the absolute number of follicles per female [18], but is adequate to assess relative differences between treatment groups. All procedures were approved by the Animal Care and Use Committee of the University of California, Berkeley.

Statistical Analyses

Body mass data from weaning until 40 wk of age were analyzed using ANOVA for repeated measures (rANOVA). Group differences at individual time points were assessed using between-subjects ANOVAs, followed by Fisher protected least significant difference test when significant. We defined stable body mass as a period during which body mass changed less than 0.5 g/wk for at least eight consecutive weeks. An ANOVA was used to analyze data for age and body mass at the time of vaginal patency. Uterine and paired ovarian weights were analyzed using the Student *t*-test or the Median test, the latter being used when large differences in variance could not be corrected by transformation of data [19]. Chi-square and Fisher exact test were used to analyze litter and weaning success data; success was defined as at least one live pup. Observed differences were considered statistically significant if $P < 0.05$, and these are reported as such regardless of the actual *P*-value.

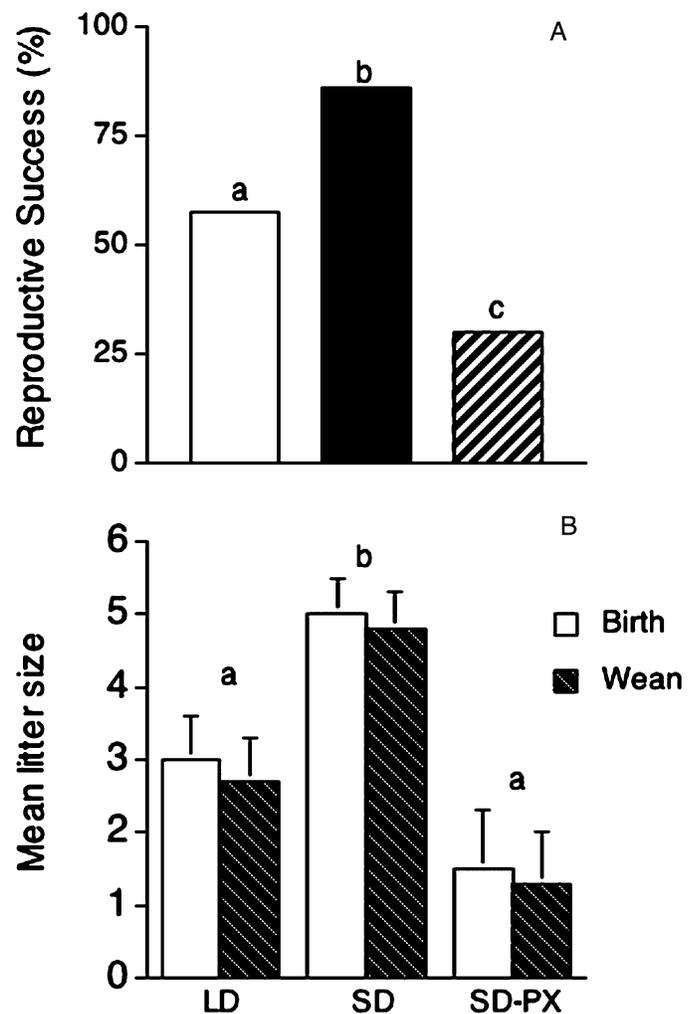


FIG. 1. A) Reproductive success of LD, SD, and SD-PX females expressed as the percentage of females mated at 9 mo of age that delivered live litters. Note: All females that littered successfully weaned at least one pup, thus litter and weaning success were identical. Different letters denote pairwise comparisons of reproductive successes that were significantly different among the three groups ($P < 0.05$). B) Mean \pm SEM live litter size at birth and weaning. Same designations as in Figure 1A.

RESULTS

Effects of Photoperiod and Pinealectomy on Reproductive Outcomes

Fifty-eight of 60 females copulated with males, based on the presence of postcopulatory vaginal plugs and/or signs of pregnancy (pups or implantation scars). Pregnancy rates were similar across groups (Table 1). Photoperiod did, however, exert a significant effect on the outcome of mating; a higher percentage of SD compared with LD females littered successfully (Fig. 1A; $P < 0.05$). Neonatal pinealectomy adversely affected reproductive outcome; only 30% of SD-PX females littered successfully after mating ($P < 0.05$ for comparison with SD and LD groups). The higher litter success rate in SD females resulted in a significantly larger mean litter size than in LD- and SD-PX females at birth and at weaning (Fig. 1B; $P < 0.05$).

In the females showing signs of pregnancy, 100% of SD females littered successfully, but only 68% of LD and 43% of SD-PX females produced viable offspring. Differences in pregnancy success were primarily due to maternal mortality, stillbirths, or fetal resorption (Table 1). The three

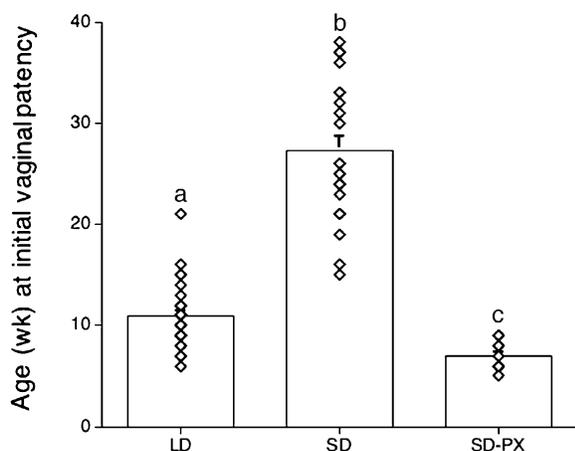


FIG. 2. Mean \pm SEM age (weeks) at initial vaginal patency, with values for individual hamsters indicated for each group. Ages at pubertal onset were significantly different among the three groups ($P < 0.05$). Note: three SD females first manifested vaginal opening after transfer to the LD photoperiod at 36 wk of age.

maternal deaths (one SD-PX and two LD females) occurred during parturition, with fetuses found entrapped within the uterine corpus or vagina. A second SD-PX female would likely have perished; this hamster was euthanized in moribund condition after delivering two stillborn fetuses, with a third lodged in the breech position within the vagina.

In dams that produced live litters, the number of live pups on the day of birth and at weaning did not differ significantly among the three groups (data not shown). Mean pup mass was nearly identical across groups on the day of birth and did not differ significantly at weaning. However, to accommodate for differences in litter size at weaning, we conducted an additional analysis limited to the modal litter size of six to seven pups; SD females weaned pups that were significantly heavier than pups of LD females (mean \pm SEM pup mass: LD male 15.3 ± 0.2 g, LD female 14.2 ± 0.2 g, SD male 16.3 ± 0.2 g, SD female 15.3 ± 0.3 g; $F_{1,112} = 19.6$, $P < 0.05$).

Developmental Effects of Photoperiod and Pinealectomy

Puberty. Vaginal patency was detected in LD females at 10.9 ± 0.6 wk and almost 4 wk earlier in SD-PX hamsters (Fig. 2; $P < 0.05$). At 13 wk of age, the vagina was closed and the uterus undeveloped in SD females, whereas LD females had patent vaginas and enlarged uteri (Fig. 3, A and D). Uterine mass and paired ovarian mass were significantly lower in SD (uterus 10.9 ± 1.4 mg; ovaries 3.8 ± 0.7 mg) than LD females (uterus 125.1 ± 27.4 mg, ovaries 8.7 ± 0.9 mg) at 13 wk ($P < 0.05$), with corresponding differences in uterine and ovarian histology (Fig. 3, B, C, E, and F). All LD and SD individuals sampled at 26 wk of age had an open vagina and uterine and paired ovarian masses that were not statistically different (not illustrated). However, the number of ovarian primordial follicles was two-fold greater in SD than LD females at both 13 and 26 wk of age (Fig. 4). Corpora lutea were present in ovaries from LD but not SD females at 13 wk of age and present in both LD and SD hamster ovaries at 26 wk of age.

Body mass. After weaning, body mass increased rapidly in LD and SD-PX females and far more slowly in SD hamsters (Fig. 5). Stable adult body mass was higher in SD-PX than LD females ($P < 0.05$ at Weeks 31–40) and was attained at 31 and 17 wk, respectively ($P < 0.05$). The slower

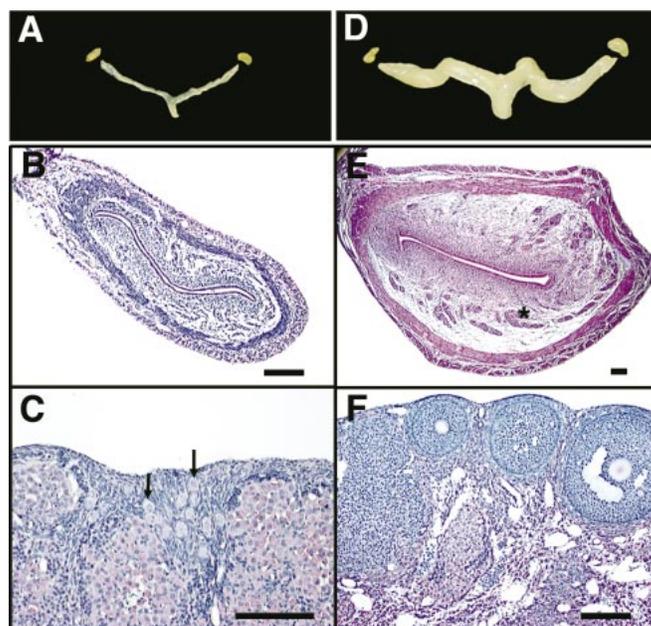


FIG. 3. Uterine and ovarian gross anatomy and histology in 13-wk-old Siberian hamsters maintained in either an SD photoperiod (A–C) or LD photoperiod (D–F). Infantile uterus of SD females is evident by narrow diameter and absence of endometrial glands (B), which are present (*) in uteri of LD females (E). Ovaries of SD females, as compared with LD females, were characterized by a greater number of primordial follicles, often found in clusters (arrows in C), and an abundance of large eosinophilic cells in the medulla. Bar = 50 μ m.

increment in body mass of SD females eventually resulted in a stable plateau at Week 6 that was maintained for the next 9 wk. A secondary increase in body mass was evident in SD females between Weeks 16 and 26, eventuating in similar body masses in LD and SD females by Week 26. After a secondary plateau from Weeks 27 to 35, body mass of SD females increased after they were transferred from the 10L to the 14L photoperiod at 36 wk of age ($P < 0.05$ for Week 36 vs. Week 40 values). When females were paired with males at 40 wk of age, mean body mass of the SD females was intermediate to that of LD and SD-PX

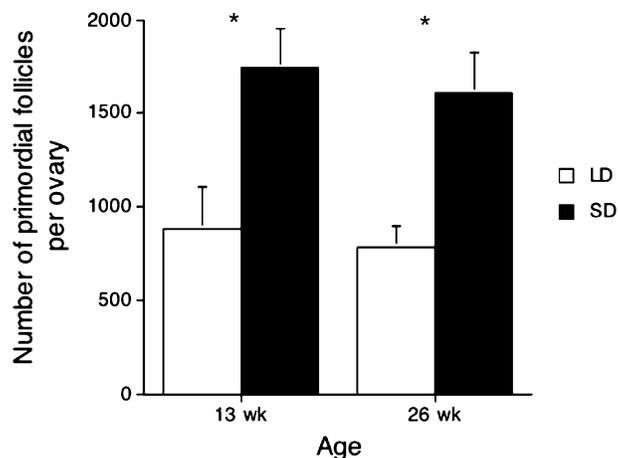


FIG. 4. Mean \pm SEM number of primordial follicles per ovary in SD and LD females at 13 and 26 wk of age. Numbers are approximations calculated by counting the number of follicles in every 12th section and multiplying by 12. Asterisk (*) denotes significant effect of photoperiodic treatment at both ages.

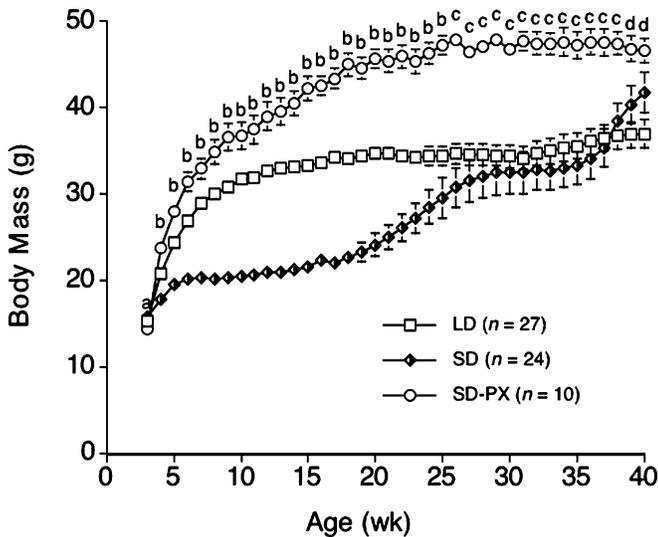


FIG. 5. Mean \pm SEM body mass (grams) of LD, SD, and SD-PX females from weaning at Postnatal Day 18 to 40 wk of age. SD and SD-PX females were transferred from a 10L photoperiod to a 14L photoperiod at 36 wk of age in preparation for mating with 14L males. Letters indicate significant differences ($P < 0.05$) between groups at individual time points: (a) SD \approx LD $>$ SD-PX; (b) SD $<$ LD $<$ SD-PX; (c) SD \approx LD $<$ SD-PX; and (d) SD \approx LD & SD-PX, but LD $<$ SD-PX.

females and not statistically different from that of either of these groups.

DISCUSSION

Short Days: Protective Effects on Reproduction at an Advanced Age

The decline in reproductive success, characteristic of middle-aged hamsters, was counteracted when females were maintained from the time of conception until 36 wk after birth in short DLs. Owing to the potent inhibitory effects of short DLs on reproductive physiology in Siberian hamsters, SD females would have had many fewer estrous cycles than LD and SD-PX females. Maintaining an enlarged, hormonally primed uterus for an extended period of time may have predisposed LD and SD-PX females to reproductive failures associated with uterine dysfunction (maternal deaths during parturition and stillborn litters). A similar phenomenon has been described in pregnant women of advanced maternal age (defined as ≥ 35 yr); older women who conceive and carry to term still face increased risks associated with dysfunctional labor (e.g., Cesarean delivery, stillbirths) [20].

Siberian hamsters, in common with other temperate zone rodents, have extended breeding seasons [4, 10]. In such species, young born several weeks after the summer solstice overwinter without achieving adult status [5]; somatic growth and reproductive maturation are delayed until the following spring when breeding begins. Because the great majority of adults from the preceding year do not survive the winter, animals born late in the preceding breeding season are essential for maintenance of the population [5, 21]. In this context, the delayed reproductive aging in Siberian hamsters born at the end of the summer, when DL has decreased well below early summer values [10], should promote reproductive success and would therefore have direct adaptive consequences [6]. At the time of initial mating the late-born cohort is up to 7 mo older than females born earlier in the year [5, 8]. Late-born females would be re-

productively compromised were it not for the protective actions of short DLs. Thus, the extension of fecundity in SD is an important component of life history fitness [21].

The progression of decreasing DLs after the summer solstice, and the correlated decrease in ambient temperature (T_a) [10], may augment and accelerate seasonal responses compared with those elicited by static short DLs and moderate T_a [22, 23]. Free-ranging hamsters born in late summer experience very low T_a during the winter [10] and deploy torpor several times each week [24], during which body temperature (T_b) declines from 37°C to 20°C. Longevity is positively correlated with the amount of time hamsters are torpid [25], and low T_b may also protect hamsters from age-related reproductive decrements.

Follicular Maintenance: Mediating Mechanisms

The number of ovarian primordial follicles, which was twice as great in SD than LD females, persisted through at least 6 mo of age. Food restriction regimens that extend reproductive life-span are correlated with decreased rates of ovarian follicular depletion (e.g., follicular reserves of calorically restricted [CR] mice 12.5 mo of age were twice those of age-matched mice fed ad libitum) [26]. Because follicular renewal has recently been described in the postnatal mammalian ovary [27], the larger follicular reserves in SD hamsters and CR mice and rats may be due to increased meiotic activity, decreased atresia, or a combination of the two. Although LD females would be predicted to undergo many more ovulations than SD females, this would not explain the large difference in primordial follicle numbers between the groups. Most ovarian follicles undergo atresia well before ovulation, and follicular atresia occurs in the presence and absence of ovulatory cycles [28].

Oocyte quality, which is negatively influenced by advancing age, profoundly affects fertility and pregnancy outcome [29]. The ovaries of SD female hamsters were developmentally younger in terms of primordial follicle number. Whether the oocytes contained therein are qualitatively superior to those of LD females remains to be determined.

Ovarian folliculogenesis begins on approximately Postnatal Day 8 in Siberian hamsters [30], which suggests DL influences follicle number after birth. However, the number of ovarian primordial follicles is also influenced by prenatal mitotic activity and rate of atresia of oogonia [28]. The relative contributions of pre- and postnatal DLs to changes in primordial follicle number remain to be established. Females in the present study were gestated and maintained postnatally in their respective photoperiods. DL influences reproductive parameters both pre- and postnatally in Siberian hamsters. DL information is communicated to fetuses via maternal melatonin secretion [31–33]. The duration of nocturnal melatonin secretion, which differs between LD and SD pups by the third week of life, also affects rates of reproductive development [34, 35].

Female Siberian hamsters maintained in SD weigh less than their LD counterparts [11, 12, and the present study] and consume less food [36]. Based on the timing of weight gains, we estimate that food intake is reduced for ~ 15 wk in short DLs. This self-imposed caloric restriction does not exceed 16% [36], but may nevertheless influence ovarian development. Food-restricted rats retain greater numbers of primordial ovarian follicles even after extended refeeding that restores body mass [37]. A similar phenomenon may explain the increased follicle number in SD hamsters at 26

wk of age, at which time their body masses were not significantly different than those of LD females (Fig. 5).

The changes in food intake in SD female Siberian hamsters differ in several important respects from those effective in extending reproduction in other species. Mice and rats usually are maintained for many months on rations that provide 20%–50% fewer calories than normal [26, 38] and often sustain body mass losses of up to 50% [39]. By contrast, SD hamsters were never calorically restricted; their food intake was reduced over the course of treatment by ~5%–10% relative to consumption by LD animals [40]. This decrease was voluntary (i.e., it occurred although animals were fed ad libitum). Reductions in food intake and decreases in body mass as DL decreases are adaptive in diminishing energy demands during winter foraging [40].

Decelerated reproductive aging may be causally related to the substantial delay in puberty in SD Siberian hamsters [e.g., 11, and the present study]. The reproductive life span also was extended by several months in mice genetically selected for delayed puberty [41]. Whether the absolute duration of reproductive life span is increased in SD hamsters with late onset reproduction, or merely shifted to advanced chronological ages, is presently uncertain.

The suppression of estrous cycles during the first 4 mo of life in SD hamsters resulted in reduced exposure to estrogens, as evidenced by delayed uterine maturation [12, present study] and lower blood concentrations of estradiol [42] in SD versus LD females. Ovarian estrogens contribute to reproductive senescence [43]. Age-related neuroendocrine and uterine changes are retarded or prevented by long-term ovariectomy and accelerated by estrogen treatment [44, 45] (e.g., reductions in plasma estradiol concentrations delay the reproductive decline in older female rats [46]).

Melatonin: Endocrine Representation of Day Length

The duration and amplitude of nightly melatonin secretion is greater in SD than LD female Siberian hamsters [12, 47], and it is plausible that melatonin directly ameliorates reproductive decline in middle-aged hamsters. Short-term melatonin treatment completely reversed the decrease in GnRH expression with advancing age in rats [48]. Melatonin treatment also preserved basal concentrations of pituitary hormones and pituitary responsiveness of old rats [49] and delayed the termination of estrous cycles in older female mice, without influencing food consumption [50]. Despite its association with delayed reproductive senescence, melatonin treatments have not increased retention of primordial follicles in aging animals [18].

Pinealectomized females housed in short DLs achieved puberty substantially earlier and maintained higher body masses than females housed in the long (14 h) DL. Siberian hamsters respond to DL in a graded fashion over a range of DLs [51]. Pinealectomy constitutes a photoperiodic signal of its own [52], which in the present context appears to be significantly longer than 14 h. The reduced reproductive success of pinealectomized middle-aged hamsters (Table 1) may be a consequence of direct changes in the hypothalamic-pituitary-gonadal axis from prolonged estrogen exposure and/or behavioral changes associated with the withdrawal of melatonin [53].

This study demonstrates for the first time that an abiotic environmental signal (i.e., photoperiod) elicits a suite of physiological responses that decelerate reproductive senescence in a female mammal. Photoresponsive species, which naturally generate seasonal cohorts of females that age re-

productively at different rates, provide useful animal models to investigate environmentally controlled aging. Study of such species [e.g., 10] will complement the genetically based models that currently dominate aging research [54, 55].

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