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Short Photoperiod Initiated During Adulthood Sustains Reproductive Function in Older Female Siberian Hamsters More Effectively Than Short Photoperiod Initiated Before Puberty[1]

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ABSTRACT

Reproductive aging in female mammals is characterized by a progressive decline in fertility and fecundity. Many women delay their first full-term pregnancy until an age at which their reproductive potential has already declined. No treatment is presently available to delay the aging process. In a limited number of rodent species, caloric restriction sustained reproductive function in older females, and in most investigations, sexual maturation was delayed because caloric restriction was initiated at weaning. We have previously reported similar outcomes in female Siberian hamsters that were reared in short photoperiod (SP), which profoundly inhibits reproductive physiology. When compared to hamsters held in long photoperiod (LP), females reared in SP matured much later and had greater reproductive success at 9 mo of age. Herein, we determined if delayed onset of sexual maturation was necessary for SP to decelerate reproductive aging. We initiated a 6-mo period of SP before or after sexual maturation and measured the reproductive success of females at 12 mo of age. Maintenance of hamsters in SP beginning after puberty was associated with significantly greater litter success (77%) compared to imposition of SP before puberty (35%); the difference in weaning success was even greater (73% and 12%, respectively). Regardless of which SP regime was used, litter success of females exposed to SP was substantially greater than that of 12-mo-old females held continuously in LP (6%). The efficacy of SP in decelerating female reproductive aging is manifest at several life stages and is greater when treatment is initiated after rather than before puberty.

aging, hamster, photoperiod, puberty, reproductive

INTRODUCTION

The decline in reproductive function associated with aging in female mammals is well known, but successful efforts to decelerate or delay this process have been limited to a modest number of species and regimens. Mice and rats have been the subjects of most mammalian investigations involving caloric restriction [1–4], and mice are the favored species for genetic manipulations [5]. In many studies [1–3], caloric restriction was initiated at or before weaning, which resulted in stunted postweaning growth and a delay in sexual maturation. This leaves open the question as to the importance of pubertal delay in postponement of the inevitable decline in reproductive function. Earlier studies have demonstrated that a delay in the timing of sexual maturation is not necessary for caloric restriction to sustain reproductive function in female rats and mice of advanced chronological age [3, 4], but to our knowledge, no single study on female reproductive aging has directly compared a regimen that was initiated before and after sexual maturation. The present study addresses this issue.

The Siberian hamster (*Phodopus sungorus*) was selected for investigation, because the rearing of females in short photoperiod (SP) significantly delays the onset of sexual maturation relative to hamsters held continuously in long photoperiod (LP) and does so when SP is initiated at or before weaning [6–8]. SP also profoundly inhibits the hypothalamic-pituitary-gonadal axis of adult hamsters [9, 10]. To date, however, the effects of SP on hamster fertility and fecundity have only been assessed when SP was initiated before the onset of sexual maturation [7]. Thus, the relative importance of delayed puberty versus an extended period of reproductive quiescence for the modulation of female reproductive aging remains to be determined. In Siberian hamsters, reproductive physiology can be inhibited before and after sexual maturity by initiating exposure to SP at different ages.

Siberian hamsters are seasonal breeders and restrict their reproductive effort from spring to late summer [11]. Females born into long or increasing day lengths (spring or early summer) mature rapidly and breed in the year of their birth. Females born in the midsummer are likely to mature quickly and then suppress reproductive activity until the following spring [12]. When females are born into short or decreasing day lengths (late summer), they delay postweaning growth and reproductive development until the following spring. Thus, females born toward the end of the breeding season will mate for the first time at 7 mo of age or older. The developmental trajectory of female hamsters reared in SP in the laboratory mimics that of pups born late in the breeding season in the field. Delayed puberty and slowed somatic growth are thought to be adaptive in the context of dwindling energy supplies and increasing thermoregulatory demands [13]. Inhibition of reproductive physiology by day length is mediated by the neuroendocrine response to the SP melatonin signal, which results in lower circulating concentrations of gonadotropins [14, 15] and greater sensitivity to the negative feedback effects of sex steroids [16].

The hypothesis tested in the present study was that SP initiated after sexual maturation in Siberian hamsters would be no better than SP initiated before puberty in sustaining reproductive function in older females. We anticipated SP after puberty might be less effective than SP before puberty,

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TABLE 1. Group designations for experimental females.[a]

	Day lengths during 3-mo age intervals			
Groups[b]	0–3 mo	3–6 mo	6–9 mo	9–12 mo
SP-LP	**10L**	**10L**	16L	16L
LP-SP-LP	16L	**10L**	**10L**	16L
LP	16L	16L	16L	16L

[a] Boldface indicates time spent in SP for the SP-LP and LP-SP-LP females. Each female underwent its first and only mating test at 12 mo of age.
[b] SP-LP females were raised in 10L and transferred to 16L at 6 months of age. LP-SP-LP females were raised in 16L and transferred to 10L at 3 months of age, where they remained until 9 months of age, when they were transferred back to 16L. LP hamsters were held in 16L throughout.

because the size of the ovarian primordial follicle pool is significantly smaller in LP-reared than in SP-reared hamsters by 3 mo of age [7]. This was the age at which young adult hamsters in the present study were transferred from LP to SP. By 12 mo of age, the initiation of SP treatment relative to the timing of sexual maturation does not affect the number of ovarian follicles [17]. Thus, SP after puberty might be just as effective as SP before puberty in sustaining reproductive function in older females. Entering into the present study, we had no indication that initiating SP after puberty would be more effective than treatment initiated before puberty, as the result of a recent caloric restriction study of mice suggested [4]. To test our hypothesis, we exposed hamsters to SP for 6 mo, beginning either before or after sexual maturation (refer to Table 1 for group designations), and paired them with males for the first time at 12 mo of age. We compared the fertility and fecundity of the SP-exposed groups to each other and to that of 12-mo-old females held continuously in LP. Separate cohorts of LP females underwent a mating test at either 4 or 8 mo of age to assess the age-associated decline in reproductive function when *P. sungorus* females are maintained in LP.

MATERIALS AND METHODS

Animals

Siberian hamsters from our colony (14 h of light per day) were transferred to LP (16 h of light per day) or SP (10 h of light per day) as breeding pairs to generate females for the following experiment. Experimental females were assigned to one of three groups, each with a different photoperiodic history: LP, LP-SP-LP, and SP-LP (Table 1). The time of lights-off was synchronized for all animals to 1700 h Eastern Standard Time. Animals were originally derived from wild-bred stock obtained from Dr. K. Wynne-Edwards, Queen's University (Kingston, Ontario, Canada). Hamsters were weaned on Postnatal Day 18, ear-tagged for identification, weighed, and placed in polypropylene cages (n = 2–4 siblings/cage). Food (Teklad 8626) and water were available ad libitum. Ambient temperature and relative humidity were held constant at 21 ± 5°C and 50% ± 10%, respectively. Body mass and vaginal patency were assessed and recorded weekly. Experimental procedures were approved by Cornell University's Institutional Animal Care and Use Committee and conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals.

Mating Tests

At 12 mo of age, each virgin female was transferred to a clean cage and paired with a sexually inexperienced adult male from our colony for 10 d. Females were inspected daily for a vaginal plug. Females were housed singly after removal of the male and inspected twice daily (morning and evening) for the presence of litters. On the day of parturition, numbers of live and dead pups were counted, and litter mass (±0.1 g) of live pups was recorded. Live pups were returned to the dam's cage after weighing. Females in which all pups were found dead or that failed to give birth 20 d after separation from the male were administered a lethal dose of sodium pentobarbital and exsanguinated by retro-orbital bleed. Their uteri were inspected under a dissecting microscope for fetoplacental tissue and/or implantation scars. Litters were weaned at Postnatal Day 18, and pups were individually weighed and sexed. Upon weaning, dams were killed and their uteri inspected as described above.

To document the age-associated decline in fertility in continuous LP, additional LP females underwent mating tests, as described above, at either 4 or 8 mo of age. As a result, virgin LP females underwent fertility testing at 4, 8, or 12 mo of age.

TABLE 2. Numbers of females that were paired with males for the first time at specific ages and after different photoperiodic histories, and the numbers and percentages of those paired females that mated.

	Age (mo)				
	Photoperiodic History				
	4	8	12	12	12
Females	LP	LP	LP	SP-LP	LP-SP-LP
No. paired	20	20	25	24	23
No. mated (%)*	16 (80%)	19 (95%)	18 (72%)	17 (71%)	22 (96%)

* A postcopulatory vaginal plug and/or signs of pregnancy (pups or implantation scars) were taken as evidence of mating.

Statistical Analysis

Results were analyzed with a commercial statistical program (JMP Ver 8; SAS Institute). The mean numbers of implantation sites and live offspring (day of birth and weaning) were analyzed by one-way analysis of variance (ANOVA) and Tukey-Kramer honestly significant difference post-hoc tests. Outcomes of mating tests (percentage of paired females that mated, percentage of mated females with implantation sites, and live offspring on day of birth and/or at weaning) were assessed by contingency tables and Pearson chi-square analyses, followed by correspondence analyses to determine which groups clustered together. Body mass data, recorded weekly from weaning to 52 wk of age, were analyzed by repeated-measures ANOVA. One-way factor ANOVAs were used to determine specific ages at which body mass means were significantly different. Differences at $P < 0.05$ were considered to be significant. Analysis of the vaginal patency data was limited to a descriptive graph.

RESULTS

Outcomes of Mating Tests

The numbers of females that were paired with males for the first time at specific ages and the numbers and percentages of paired females that mated are summarized in Table 2. The majority of females in all groups mated, with the highest percentages in the 8-mo LP and 12-mo LP-SP-LP groups. Group effect on the percentage of paired females that mated approached, but did not achieve, statistical significance ($P = 0.06$).

Fertility and fecundity declined between 4 and 8 mo of age in female hamsters held in LP, and the decline in reproductive function between 8 and 12 mo of age was even more pronounced in LP females (Fig. 1). Only 50% of the LP females that mated at 12 mo of age showed any signs of pregnancy, and of those, only 6% produced a viable litter. By contrast, females that had been exposed to SP and were first mated at 12 mo of age were more likely to conceive and produce viable offspring ($P < 0.05$). Moreover, by almost every measure, female hamsters first exposed to SP as young adults (LP-SP-LP females) had significantly better reproductive outcomes when mated at 12 mo of age than females reared in SP (SP-LP females) and females held in LP ($P < 0.05$) (Fig. 1). The majority of LP-SP-LP females that mated at 12 mo of age had live pups on the day of birth and at weaning, whereas a minority of age-matched SP-LP and LP dams littered or weaned successfully. The number of implantation sites was comparable in SP-LP and LP-SP-LP females, but the number of live offspring per dam on the day of birth and at weaning was significantly greater in the LP-SP-LP group ($P < 0.05$)

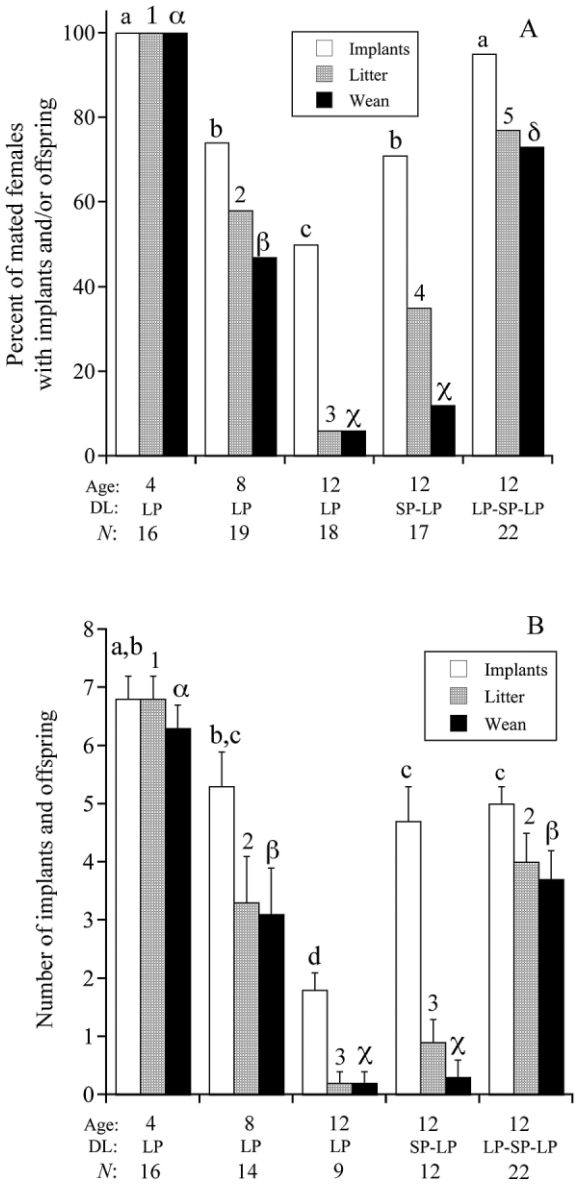

FIG. 1. **A**) Percentages of mated females with uterine implantation sites, live offspring on the day of birth, and/or live offspring at weaning. **B**) For those females that had implantation sites detected, the number (mean + SEM) of implantation sites and the number (mean + SEM) of live offspring on the day of birth and at weaning. The age (mo) at which females were first paired with males and their day length (DL) history are provided along the abscissa. Samples sizes (N) are also provided for the numbers of females that mated (**A**) and that had implantation sites detected (**B**). Bars not sharing the same letters (implants), numbers (litter), or symbols (wean) are significantly different ($P < 0.05$).

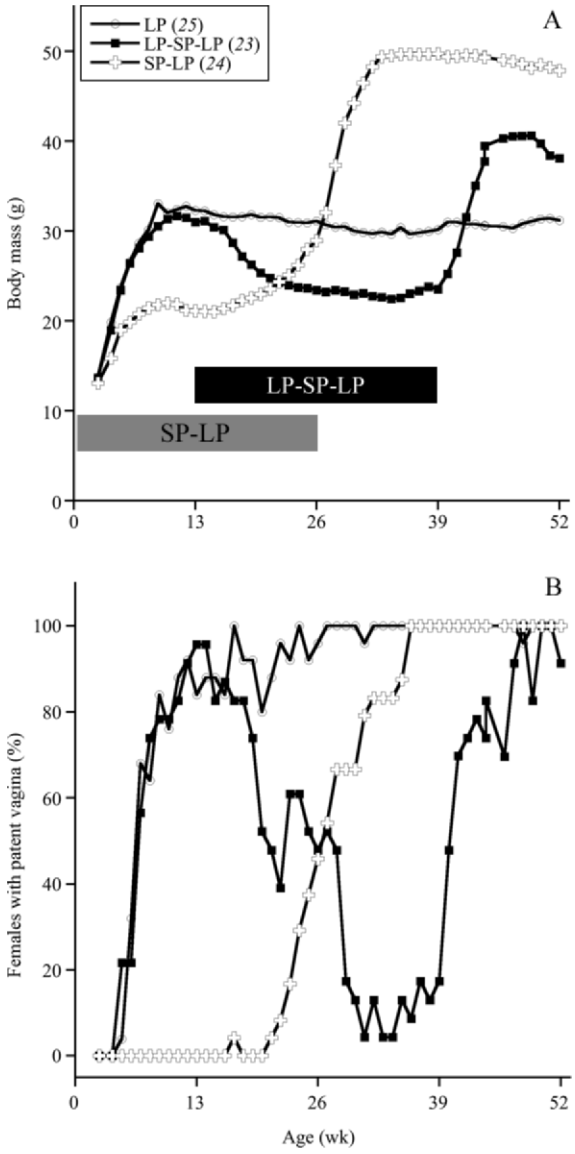

FIG. 2. **A**) Weekly mean body mass of female hamsters that were first paired with males at 12 mo (52 wk) of age. Body mass recordings began at weaning on Postnatal Day (PD) 18. Sample sizes are given parenthetically in the key. Shaded horizontal bars indicate time spent in SP. Error bars are omitted for clarity. **B**) Percentage of females with a patent vagina, recorded weekly from weaning on PD18 to 52 wk of age.

(Fig. 1B). Overall, LP females fared poorly when mated at 12 mo of age, and the outcomes for SP-LP females were intermediate between those of LP and LP-SP-LP females. With regard to the numbers of implantation sites and live offspring, the LP-SP-LP females were statistically indistinguishable from LP females first mated at 8 mo of age (Fig. 1B).

Effects of Photoperiod on Body Mass, Sexual Maturation, and Reproductive Inhibition

The SP treatment before sexual maturation was associated with decelerated postweaning growth; SP-LP females weighed significantly less than LP females by 4 wk of age (Fig. 2A). During the postweaning growth phase, the largest difference in body mass between SP-LP and LP females was at 12 wk of age, when SP-LP females weighed 35% less than LP females. SP-LP females were transferred from SP to LP at 26 wk of age, but increases in body mass preceded the change in day length, which is consistent with their becoming refractory to SP after 18–20 wk [18, 19]. However, the rate of increase in body mass accelerated upon transfer to LP at 26 wk of age, and SP-LP females were significantly heavier than females in the other groups during the last 24 wk of monitoring.

Shortly after transfer to SP at 13 wk of age, body mass in LP-SP-LP females began to decrease, and these females were significantly lighter than LP females after 4 wk of SP treatment. Body mass differences between LP-SP-LP and LP females were maximized after 13 wk in SP, at which time LP-SP-LP females weighed 25% less than LP females at 26 wk of age. LP-SP-LP

females reached their body mass nadir (22.5 g) after 18 wk in SP, at 31 wk of age. Signs of refractoriness to SP were less pronounced in LP-SP-LP than in SP-LP females, but a prompt increase in body mass upon transfer back to LP at 39 wk was evident in LP-SP-LP females (Fig. 2). Nevertheless, body mass in LP-SP-LP females plateaued at a lower weight than in SP-LP females. In contrast to females that were exposed to SP, hamsters held continuously in LP maintained a fairly stable body mass after completing the postweaning growth phase.

The first onset of vaginal patency was used as the index of sexual maturation; as expected, vaginal opening was substantially delayed in SP-LP females (26.8 ± 0.7 wk) relative to LP females (7.2 ± 0.3 wk) and LP-SP-LP females (7.4 ± 0.4 wk) (Fig. 2B). The hypothalamic-pituitary-gonadal axis is inhibited in adult hamsters transferred from LP to SP, and the average time to vaginal closure in LP-SP-LP females occurred after 10.0 ± 1.2 wk in SP. The mean duration of vaginal closure in these adults was 16.4 ± 1.1 wk. A rapid and/or prolonged response was not superior to a more modest response to SP in terms of reproductive outcomes at 12 mo of age.

DISCUSSION

The present study establishes that inhibition of reproductive physiology, in this case by exposure to SP, sustains reproductive function in older females even when the timing of sexual maturation is not delayed. Initiation of SP treatment after sexual maturation was of greater benefit to older female hamsters compared with treatment initiated before puberty. The effects of postpubertal SP in hamsters are similar to the effects of caloric restriction imposed postpubertally in female mice [4]. The present study has the added advantage of comparing treatments before and after puberty in a single investigation.

The phenotypic response to SP in Siberian hamsters includes a decline in body mass and reduced food intake even when food is available ad libitum. To our knowledge, the effects of the voluntary reduction in food intake on reproductive aging in hamsters have not been investigated in isolation from the other effects of SP; that is, hamsters in LP have not been fed a diet to match the caloric intake of females in SP. It remains unknown if the intensity and duration of reduced food intake in SP is sufficient to modulate the decline in fertility and fecundity associated with reproductive aging. Food intake was not monitored in the present study, but the body mass of SP-LP and LP-SP-LP females while in SP was as low as 66% and 76%, respectively, of the body mass of LP females. These photoperiod-induced differences in body mass in hamsters are comparable in scope to those achieved by caloric restriction in mice and rats [2–4, 20–22]. The caloric restriction regimens used in those studies were associated with a deceleration of reproductive and/or general aging. To sustain body mass losses to this level, rations fed to mice and rats were reduced to 20–50% of those consumed by conspecifics fed ad libitum. Adult Siberian hamsters transferred from SP to LP, on the other hand, voluntarily reduce food intake by 10–16% relative to animals held in LP [23, 24]. For hamsters reared in SP, in a separate study we estimated food intake in SP was approximately 22% less than food intake in LP at 8 wk of age (unpublished data). Thus, the level of caloric restriction associated with SP in Siberian hamsters may be at the lower end of the effective range used in other rodents. However, because most hamsters become refractory to SP after 18–20 wk, the duration of reduced food intake for hamsters in SP is generally shorter than the period of caloric restriction imposed upon mice and rats [2–4, 20–22]. Interestingly, in a study of longevity, Yu et al. [25] found rats maintained on reduced rations lived significantly longer than rats fed ad libitum, whether caloric restriction was initiated at 6 wk or 6 mo of age. However, when caloric restriction was limited to the period between 6 wk and 6 mo of age, the increase in longevity was minimal. Reduced food intake per se by hamsters in SP may be of insufficient duration to sustain reproductive function in older females.

No clear explanation exists as to why initiating SP after puberty preserved reproductive function in older female hamsters more effectively than SP treatment before puberty. Assessment of ovarian follicle numbers failed to uncover a difference between SP-LP and LP-SP-LP females at 12 mo of age [17]. The period of SP-induced reproductive quiescence occurred nearer to the time of mating in LP-SP-LP than in SP-LP females, but the importance of the temporal relation between the inhibited and active reproductive states remains to be elucidated. Even though the time spent in SP was the same (6 mo) for SP-LP and LP-SP-LP, the SP-LP females as a group may not have garnered the full benefit of those 6 mo, because many individuals became unresponsive to short day lengths (photorefractoriness). About half (54%) of the SP-LP but more than 80% of LP-SP-LP females still had closed vaginas at the end of SP treatment, which ended 3 mo before mating tests. The poorer mating outcomes in SP-LP females may be related to the pronounced weight gain these females demonstrated after the transition from SP to LP. When paired with males at 12 mo of age, the body mass of SP-LP females exceeded that of LP-SP-LP females by almost 10 g (~26%). With an average body mass of 48 g, a pear-shaped body, and abundant intra-abdominal fat, the SP-LP females appeared to exhibit adult-onset obesity in response to the transfer to LP. Because obesity negatively impacts fertility [26–30], some of the benefits of SP-induced delayed sexual maturation in SP-LP females may have been negated by rapid and sustained weight gain in LP. In a model of premature reproductive aging in mice that is closely related to adult-onset obesity, age- and obesity-associated changes in ovarian gene expression were noted in signaling pathways involving glucocorticoid metabolism, leptin, and insulin [30]. Whether these changes in ovarian gene expression are present in obese hamsters remains to be determined, as do their effects on fertility.

In conclusion, photoperiodic history is a strong modulator of female reproductive aging in Siberian hamsters. This study adds to a growing body of literature suggesting that various treatments initiated in adulthood effectively extend reproductive function and/or longevity [4, 31, 32]. The effects of SP on longevity in hamsters remains to be determined, but given the parallels between the effects of caloric restriction on reproductive function and longevity in mice and rats, it seems plausible that hamsters exposed to SP will live longer than hamsters held continuously in LP. Because the initiation of SP relative to sexual maturation had a substantial effect on reproductive function in older females, it will be important to determine if the timing of SP exposure relative to puberty similarly affects longevity.

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