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Graded response to short photoperiod during development and early adulthood in Siberian hamsters and the effects on reproduction as females age

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ABSTRACT

Short day (SD) lengths delay puberty, suppress ovulation, inhibit sexual behavior, and decelerate reproductive aging in female Siberian hamsters (*Phodopus sungorus*). To date, the modulation of the age-associated decline in reproductive outcomes has only been demonstrated in female hamsters experiencing different day lengths during development. To determine if developmental delay is necessary for photo-inhibition to decelerate reproductive aging, hamsters raised in LD were transferred to SD as young adults and remained there for 6 months. Females that demonstrated the most immediate and sustained photo-inhibition were found to have greater numbers of ovarian primordial follicles at advanced ages (9 and 12 months) than did females held in LD, nonresponders to SD, and females with a marginal SD-response. Similarly, for females raised in SD from conception to 6 months of age, prolonged developmental delay was associated with greater numbers of primordial follicles at later ages as compared to hamsters that became refractory to SD. A robust response to SD in juvenile and adult hamsters is associated with decelerated reproductive aging, which may result in greater reproductive success in older females as compared to age-matched individuals demonstrating a more modest response to SD.

Introduction

The life history strategy of seasonally breeding rodents is strongly influenced by the photoperiodic conditions at the time of birth and during development. Young born before or near the summer solstice mature quickly, and females are likely to produce offspring in the year of their birth (Bronson, 1985). Conversely, when the breeding season extends past the summer solstice into late summer and early autumn, as day length decreases, young individuals are more likely to delay puberty and defer their first reproductive effort until the following spring (Butler et al., 2007).

In Siberian hamsters, *Phodopus sungorus*, raising females in short days (SD) was associated with a delay in reproductive aging (Place et al., 2004). Decelerated reproductive aging in SD females, as compared to females held in long days (LD), was manifested as greater reproductive success when LD and SD females were first paired with males at 9 months of age. Additionally, SD females had significantly more ovarian primordial follicles than LD hamsters at 3 and 6 months of age (Place et al. 2004), and this advantage persisted through 12 months of age, even though SD females had been transferred to a long photoperiod 4 months prior (unpublished results). Primordial follicles represent the resting pool of germ cells in the mammalian ovary, and their numbers decline with age because activation of follicular growth is irreversible and occurs throughout a female's lifetime (Zuckerman, 1951; vom Saal et al., 1994). In women, when ovarian follicles are completely depleted, or nearly so, the menopause ensues.

Because puberty is significantly delayed in Siberian hamsters raised in SD, the deceleration of reproductive aging may reflect a shift in their life history trajectory that results from delayed maturation. However, hamsters transferred to SD as adults also demonstrate a profound inhibition of reproductive physiology — gonadotropin levels are suppressed relative to LD hamsters (Dodge and Badura, 2002; Kenny et al., 2002), ovulation ceases, the uterus decreases in size, and the vaginal opening closes (Lerchl and Schlatt, 1993; Schlatt et al., 1993). To determine if SD after puberty affects reproductive aging, we compared outcomes in older female hamsters held continuously in LD to that of females exposed to 6 months of SD either before or after puberty. If the attrition of primordial follicles is not tempered by SD exposure after puberty, then pubertal delay assumes greater importance in the previously described account of SD-induced deceleration of reproductive aging (Place et al., 2004). This finding would also suggest that females in the wild that mature during the year of their birth, may experience continued reproductive decline even if they respond to decreasing day length and suppress their reproductive physiology. As such, if they survive to the next spring's breeding season their capacity to breed successfully may be reduced, especially compared to overwintering females that delayed puberty (Schwarz et al., 1964).

In addition to counting ovarian follicles, serum concentrations of anti-Müllerian hormone (AMH) were also measured because this

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hormone has been found to reflect the size of follicular pool in mice (Kevenaar et al., 2006) and in women (van Rooij et al., 2005). AMH is produced by ovarian granulosa cells of growing follicles (Baarends et al., 1995), but we have also noted its expression in hypertrophied granulosa cells that surround atretic oocytes in ovaries from Siberian hamsters raised in SD (Kabithe and Place, 2008). These hypertrophied granulosa cells have a luteinized histological appearance, and they occupy a large majority of the ovarian volume in SD hamsters, which appears to account for the three-fold higher levels of AMH in SD as compared to LD hamster ovaries (Kabithe and Place, 2008). Moreover, AMH inhibits primordial follicle activation in mice (Durlinger et al. 1999, 2002), and thus higher AMH in the ovaries of hamsters held in SD may manifest as greater numbers of primordial follicles.

The physiological responses to short or decreasing photoperiod go beyond inhibition of the reproductive axis, as Siberian hamsters in SD manifest differences in food intake, body mass, and pelage (Lerchl and Schlatt, 1993, Knopper and Boily, 2000). These reactions to changing environmental conditions are thought to increase the chances of overwinter survival as resources diminish and energetic demands of foraging and temperature regulation increase (Dark and Zucker, 1985). Similarly, modulation of the immune system by photoperiod has been observed in Siberian hamsters (Bilbo et al., 2002; Prendergast et al., 2004; Demas and Sakaria 2005; Weil et al., 2006), which has been postulated to be adaptive in the context of seasonally variable immune challenges and energetic demands (Prendergast et al., 2001). The T-cell mediated antibody response to a naïve antigen (keyhole limpet hemocyanin, KLH) has been reported to be lower in SD than in LD hamsters (Yellon et al., 1999; Drazen et al., 2001; Demas, 2002), but to date all studies have evaluated relatively young adults. In the present study we assessed the antibody response to KLH challenge in hamsters ranging from 3 to 12 months of age, to determine if the response deteriorates with age, and if so, is that age-associated decline modulated by photoperiodic history. We anticipated a lower KLH-antibody response in the oldest LD animals, because of the reported changes in T-cell subsets with age in mice (Miller, 1996), with a shift from naïve to a larger proportion of memory T-cells. Seeing as reproductive and somatic aging are so thoroughly intertwined (Williams, 1966; Stearns, 1992), we set out to determine if a photoperiodic history that decelerates reproductive aging will also modulate a potential biomarker of somatic aging.

Methods and materials

Experimental animals

Siberian hamsters from our colony (14 h of light per day, 14 L) were transferred to LD (16 L) or SD (10 L) as breeding pairs to generate females for the following experiment. Experimental females were assigned to one of three groups (Table 1). The time of lights-off was synchronized for all animals to 1700 Eastern Standard Time (EST). Animals were originally derived from wild-bred stock obtained from Dr. K. Wynne-Edwards, Queen's University. Hamsters were weaned on postnatal day 18, ear-tagged for identification, weighed, and placed in polypropylene cages (2 to 4 siblings/cage). Food (Teklad 8626, Madison, WI) and water were available ad libitum. Ambient temperature and relative humidity were held constant between 21 °C±5 and 50±10%, respectively. Body mass, coat color, 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 NRC Guide for the Care and Use of Laboratory Animals.

Table 1
Group designations for experimental females

Groups	Day lengths during 3-month age intervals			
	0–3 mo	3–6 mo	6–9 mo	9–12 mo
LD	16 L	16 L	16 L	16 L
LD–SD–LD	16 L[a]	10 L	10 L	16 L
SD–LD	10 L	10 L	16 L	16 L

LD-hamsters were held in 16 L throughout. LD–SD–LD-females were raised in 16 L and transferred to 10 L at 3 months of age, where they remained until 9 months of age, when they were transferred back to 16 L. SD–LD-females were raised in 10 L and transferred to 16 L at 6 months of age. Blood and tissues were collected from six animals from each group at 3, 6, 9, and 12 months of age. Gray shading indicates times when the LD–SD–LD- and SD–LD-females were held in SD.

[a] The photoperiodic histories of LD- and LD–SD–LD-females were identical through 3 months of age, thus in actuality there were only two groups at this sampling age.

Five to eight animals from each group were killed at 3, 6, 9 and 12 months of age to collect blood and harvest reproductive tissues (details below). Females failing to respond to SD (maintained open vagina, summer pelage, and body mass) were excluded from the 3, 6, and 9-month-old cohorts. However, a sufficient number of photo-nonresponders (NR) from within the LD–SD–LD group were available at the 12-month sampling point to include them as a fourth group. The essential study design was meant to determine if 6 months in SD results in a greater preservation of ovarian primordial follicles as compared to females held in LD, independent of the timing of the SD exposure relative to puberty.

Blood and tissue collection

Females from each group were killed at predetermined ages (3, 6, 9 and 12 mo) by intraperitoneal overdose of sodium pentobarbital and exsanguination by retro-orbital bleed. All animals were euthanized during the middle of the light cycle. Blood was clotted on ice at room temperature for 1 h and centrifuged at 3600 rpm for 20 min in 4 °C. Drawn off serum was aliquoted, frozen, and maintained at −80 °C until assayed for AMH and KLH-antibodies.

The right ovary was removed from each animal, dissected free of surrounding fat, weighed on an analytical balance, and immersed in 10% buffered formalin for histology and follicle counts. The left ovary was removed, flash-frozen on dry ice, and stored at −80 °C for another study. The uterus was then removed, dissected free of surrounding fat, and weighed on an analytical balance. Formalin fixation of the right ovary continued overnight at room temperature, followed by serial dehydration into 70% ethanol. Ovaries were embedded in paraffin and serially sectioned at 6 μm.

Quantification of ovarian follicles

Every tenth section was stained with hematoxylin and eosin (H&E; 3- and 6-month-olds) or Periodic-acid Schiff (PAS; 9- and 12-month-olds) and viewed under 400× magnification to count ovarian follicles. Because PAS staining allows for better visualization of the zona pellucida, our lab transitioned to this staining technique between the 6- and 9-month-old cohorts. A sampling of adjacent ovarian sections from 9-month-old females were stained with H&E or PAS and yielded comparable follicle counts. However, counting follicles required less time when sections were stained with PAS than with H&E, thus PAS was the method used for the older cohorts. Ovarian sections of poor histological quality were replaced with the adjacent section. For some ovaries, especially from the 6-month-old cohort, good quality replacement sections could not be found, resulting in their exclusion from the follicle count data.

Ovarian follicles were classified as primordial, transitional, primary, secondary, or antral. Primordial follicles were defined as an ooctye surrounded by a single layer of flattened granulosa cells, whereas transitional follicles contained a mix of flattened and cuboidal granulosa cells. Meredith et al., (2000) referred to transitional follicles as type B/C follicles and considered them to be part of the pool of primordial follicles, because this class of follicle consists of both slowly growing and non-growing follicles. Therefore, primordial and transitional follicles counts were combined and presented as primordial follicle counts. Primary follicles had a single layer of

cuboidal granulosa cells and secondary follicles had multiple layers. A follicle with an antrum of any size was classified as an antral follicle. To ensure that follicles were counted only once, visualization of a healthy oocyte nucleus was required for inclusion. Slides were examined by a single investigator who was unaware of the donor's treatment group.

Assay for serum AMH

AMH was measured in duplicate serum samples using an enzyme-linked immunosorbent assay (ELISA) produced by Diagnostic Systems Laboratories (Webster, TX). This ELISA was previously validated for the measurement of AMH in Siberian hamsters (Kabithe and Place, 2008); the intra- and inter-assay coefficients of variation were 22.0% and 19.8%, respectively. The minimum detection limit of the assay as reported by the manufacturer was 0.006 ng/ml.

KLH challenge and antibody determinations

Ten days before scheduled euthanasia, each animal was given a single subcutaneous injection of KLH (100 μg) suspended in 0.1 ml sterile saline. KLH is an innocuous respiratory protein derived from the giant keyhole limpet (*Megathura crenulata*) that generates a robust antigenic response in rodents, but does not make the animals sick, i.e., it does not induce prolonged inflammation or fever (Demas and

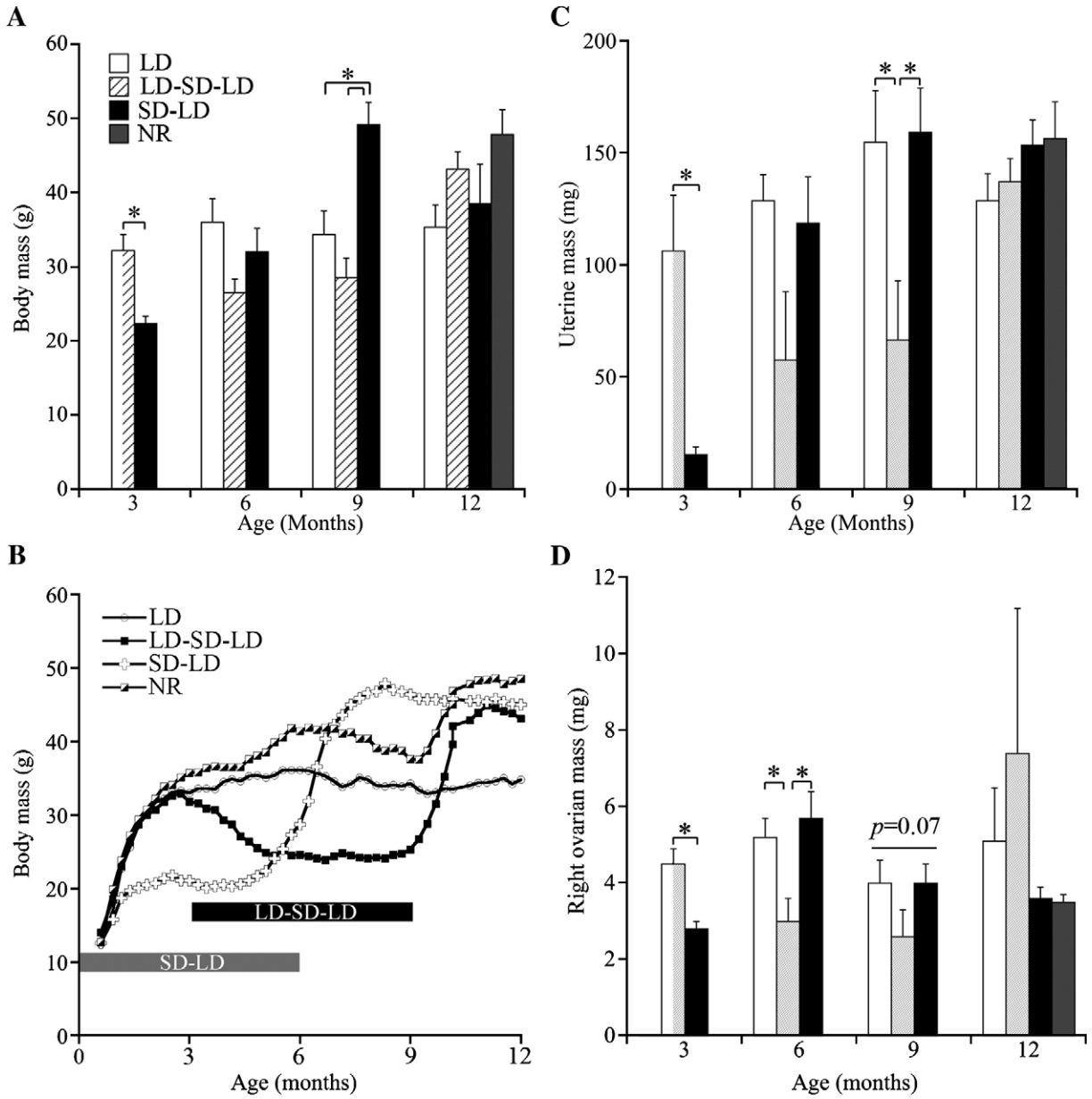


Fig. 1. (A) Mean (+SEM) body mass (g) of female hamsters at the time when animals were euthanized at 3, 6, 9, or 12 months of age. (B) Weekly mean body mass of female hamsters from the 12-month cohorts in A, recorded from weaning on postnatal day 18 to 12 months of age (52 wk). These repeated measures for each individual more clearly depict the changes in body mass as hamsters develop in different photoperiods and when they were transferred to a different photoperiod. Shaded horizontal bars indicate time in SD (10 L). Error bars were omitted for clarity. (C) Mean (+SEM) uterine mass (mg) of females as in A. (D) Mean (+SEM) right ovarian mass (mg) of females as in A. Sample sizes were five to eight animals in all groups at all ages. * denotes statistically significant differences between groups ($p<0.05$). Note: the large error bar for the 12-mo-old LD–SD–LD group was due to a large ovarian cyst in a single animal. Note: In this and all subsequent bar graphs the bars for the LD and LD–SD–LD groups at 3 months of age are combined, as the groups are represented as a single cohort of animals because of the shared photo-history from conception to this age. Group designations for this and subsequent figures: LD females were held in 16 L throughout the study; LD–SD–LD females developed in 16 L, were transferred to and held in 10 L from 3 to 9 months of age, and then returned to 16 L through 12 months of age; SD–LD females developed in 10 L and were transferred to and held in 16 L from 6 to 12 months of age; NR (nonresponder) females were a subset of hamsters from the LD–SD–LD group that maintained a patent vagina when held in 10 L.

Table 2
Percent of females in three experimental groups with patent vagina at the time points (ages in months) at which they were euthanized

Groups	Percent of females with patent vagina			
	3 mo	6 mo	9 mo	12 mo
LD	100%	100%	100%	100%
LD–SD–LD	100%[a]	20%	33%	100%
SD–LD	0%	100%	100%	100%

Gray shading indicates times when the LD–SD–LD and SD–LD females were held in SD.
[a] The photoperiodic histories of LD- and LD–SD–LD-females were identical through 3 months of age, thus in actuality there were only two groups at this sampling age.

Sakaria, 2005). Collecting blood 10 d post-immunization captures the peak IgG production during the course of the KLH immune response (Demas et al., 1997; Drazen et al., 2000).

Serum anti-KLH IgG and IgM concentrations were assayed by an ELISA previously validated in *P. sungorus* (Demas et al., 1997; Demas, 2002; Prendergast et al., 2004; Demas and Sakaria, 2005). Briefly, samples were added in duplicate to KLH-coated microtiter plates along with positive and negative controls (pooled serum from hamsters with previously determined high anti-KLH IgG or IgM levels and from hamsters injected with sterile saline vehicle, respectively), then sealed, incubated, and washed before addition of the 2° antibody (alkaline phosphatase-conjugated anti-mouse IgG or IgM). After a second wash and incubation, plates were then treated with enzyme substrate (*p*-nitrophenyl phosphate). The enzyme reaction was stopped after 20 min by adding 1.5 M NaOH and optical density (OD) was determined using a plate reader equipped with a 405 nm wavelength filter. The mean OD for each set of duplicates was calculated, and the mean OD for each sample was expressed as a percent of its plate positive control OD for statistical analyses (Demas and Sakaria, 2005).

Statistical analysis

Results were analyzed with a commercial statistical program (JMP version 7.0.2, SAS Institute, Cary, NC). The following measures required log transformation to fit a normal distribution: serum AMH concentration, right ovarian mass, primordial and secondary follicles. These variables were back transformed for the purposes of graphical representation. Homogeneity of variance was confirmed by Levene's test for all variables, with the test being utilized after log transformation when indicated. Comparisons of measures within age classes (3, 6, 9 and 12 months) were made with *t*-test or analysis of variance (ANOVA), depending on the number of groups. ANOVAs were also used to make comparisons across ages within groups, and Dennett's post-hoc test was used to determine if means at later ages were significantly different than means at 3 months of age. For the oldest groups (9 and 12 months of age), age-specific distribution analyses for the numbers of primordial follicles were completed and individuals were categorized as being 'high' if their follicle count was at or above the 75% quartile or 'low' if their follicle count was at or below the 25% quartile. Pearson chi-squared test was used to determine if the proportion of individuals falling into the high and low categories differed among groups, followed by a correspondence analysis to determine which groups clustered with the defined categories. Pearson product-moment was used to determine if serum AMH concentration correlated with the numbers of ovarian follicles. Differences at $p<0.05$ were considered to be significant.

Results

Morphometrics

Female hamsters raised in SD (SD–LD group) weighed significantly less at 3 months of age than females raised in LD, but weighed significantly more than other groups at 9 months of age (Fig. 1A). Body mass of females held continuously in LD (LD group) remained relatively stable after 3 months of age, whereas hamsters transferred to and from SD (SD–LD, LD–SD–LD, and NR groups) demonstrated more variability (Figs. 1A and B). Some SD–LD females showed signs of becoming refractory to short photoperiod before their transfer to LD at 6 months of age (Fig. 1B), but weight gain increased markedly when SD–LD females were transferred from 10 L to 16 L. Similarly, LD–SD–LD females decreased body mass when transferred to SD at 3 months of age, and then gained weight rapidly when transferred back to LD at 9 months of age (Fig. 1B). Interestingly, NR females not only failed to lose body mass when transferred to SD, but they appeared to increase their weight, and markedly so upon transfer back to LD at 9 months (Fig. 1B).

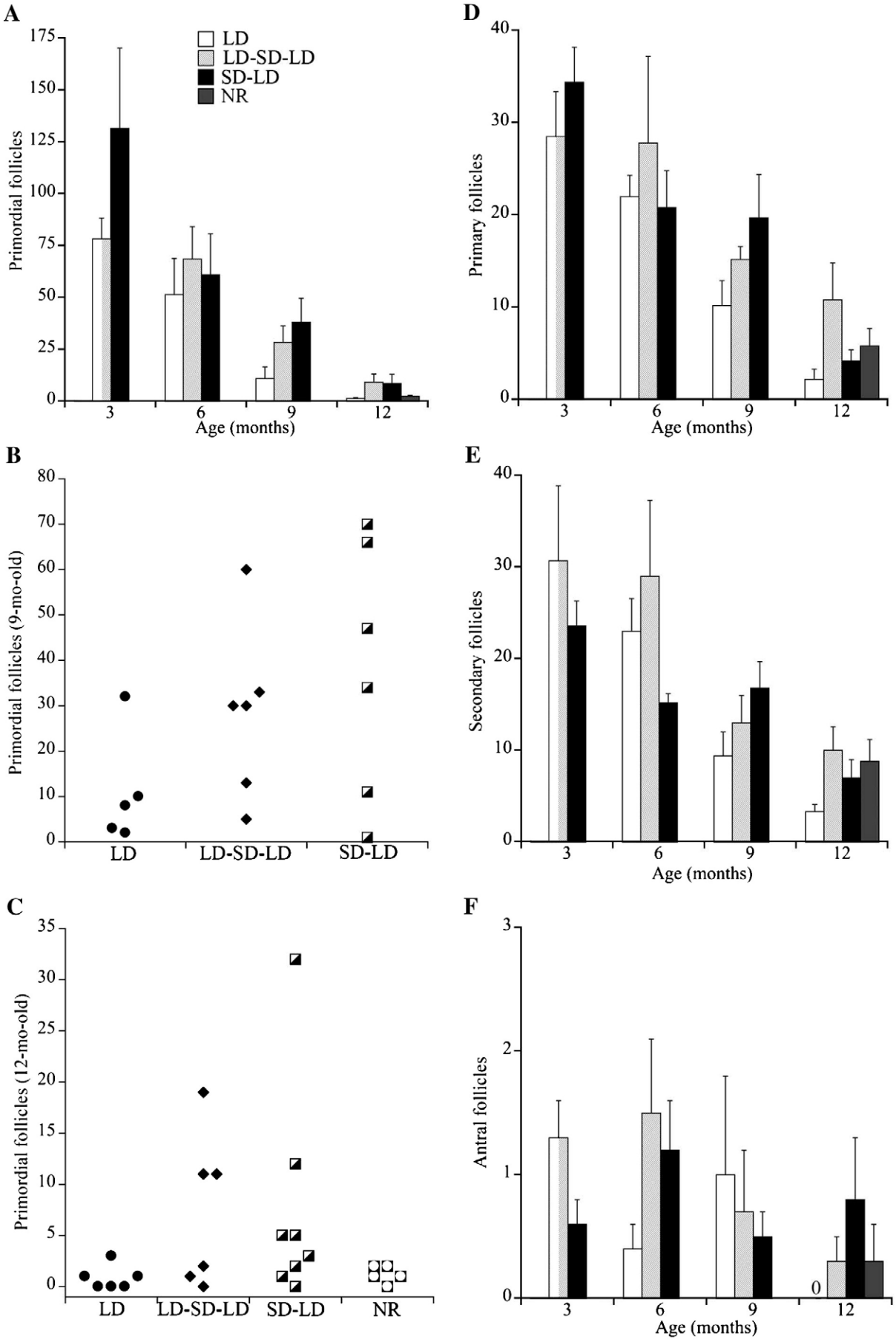
Uterine mass was lower in SD-exposed, responsive females, irrespective of the timing of SD relative to maturity (Fig. 1C). SD–LD females had significantly smaller uteri than did LD females at 3 months of age, and LD–SD–LD females had significantly smaller uteri than did LD and SD–LD females at 9 months. Uterine mass did not differ significantly between photoperiod groups at 6 and 12 months of age.

At every collection age, all LD females were noted to have a patent vagina. A closed vagina was noted in all SD–LD females at 3 months and in the majority of LD–SD–LD females at 6 and 9 months (Table 2). Note, however, that a subset of the LD–SD–LD and SD–LD females that were killed at 9 or 12 months of age did not transition from a closed to patent vagina until after they had been transferred from 10 L to 16 L — the relevance of this point will become apparent when ovarian follicle counts for these age classes are presented.

As compared to LD females, the mass of the right ovary was lower in SD-exposed, responsive females, irrespective of the timing of SD relative to maturity (Fig. 1D). Right ovarian mass in SD–LD females was at its nadir at 3 months of age, and had achieved parity with that of LD females by 6 months. Ovarian mass in LD–SD–LD females was significantly less than that of SD–LD and LD females at 6 months of age. No significant photoperiod group differences in ovarian mass were detected at 9 or 12 months of age. The apparent larger mass and variation in the LD–SD–LD group at 12 months was due to a single female having an unusually large ovarian cyst (4 mm dia.), but the inclusion or exclusion of this particular animal did not affect the outcome of the statistical analysis.

Ovarian follicles

Within the LD, LD–SD–LD, and SD–LD groups, the number of primordial follicles decreased significantly with advancing age. The decrease in the primordial follicle count was significant by 9 months of age in the LD group, whereas the change was not statistically different until 12 months of age in LD–SD–LD and SD–LD groups. Within each of the four age classes no significant differences in the number of primordial follicles were found (Fig. 2A). However, within the older age classes (9 and 12 mo), the females that had the greatest numbers of primordial follicles (at or above the 75% quartile) were significantly ($p<0.05$) more likely to be found in the groups demonstrating photo-inhibition in response to SD either as juveniles or adults (Figs. 2B and C). More specifically, at 12 months of age the primordial follicle count was four or less in all LD and NR females, whereas 50% of SD–LD and LD–SD–LD hamsters had 5 or more primordial follicles, and a number of females had substantially more (Fig. 2C). Upon completing the ovarian follicle counts, the onset and duration of each animal's response to SD was evaluated. For females raised in SD (SD–LD group), those hamsters that became refractory to SD before transfer to LD at 6 months were more likely to have fewer primordial follicles at older ages than females that maintained a closed vagina for up to 4 wk beyond the transfer to LD. Females were deemed refractory to SD if their vagina opened whilst still in SD and remained open for two or more successive weeks. Similarly, LD–SD–LD females with a delayed and relatively brief response to SD had


A
LD
LD-SD-LD
SD-LD
NR
Primordial follicles
Age (months)
B
Primordial follicles (9-mo-old)
LD
LD-SD-LD
SD-LD
C
Primordial follicles (12-mo-old)
LD
LD-SD-LD
SD-LD
NR
D
Primary follicles
Age (months)
E
Secondary follicles
Age (months)
F
Antral follicles
Age (months)

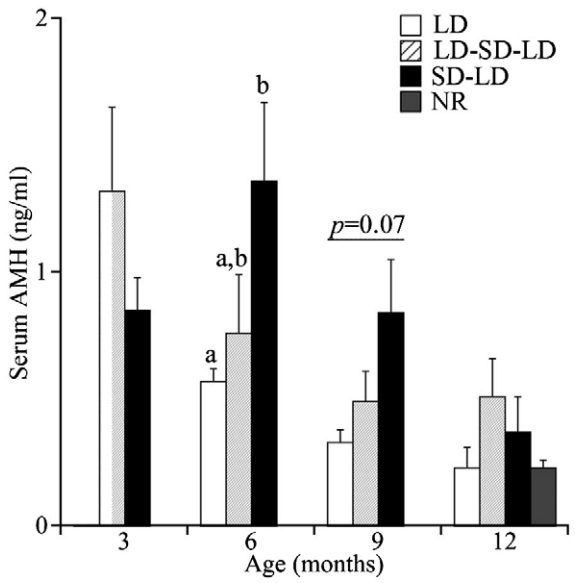


Fig. 3. Mean (+SEM) serum AMH concentrations in female hamsters at 3, 6, 9, or 12 months of age. Sample sizes were five to eight animals in all groups. Each of the photoperiod-groups (LD, LD–SD–LD, and SD–LD) showed a significant change ($p<0.05$) in serum AMH concentration with age (refer to text for details), but between-group differences were limited to the 6-month-old cohort (no shared letters indicate significant differences).

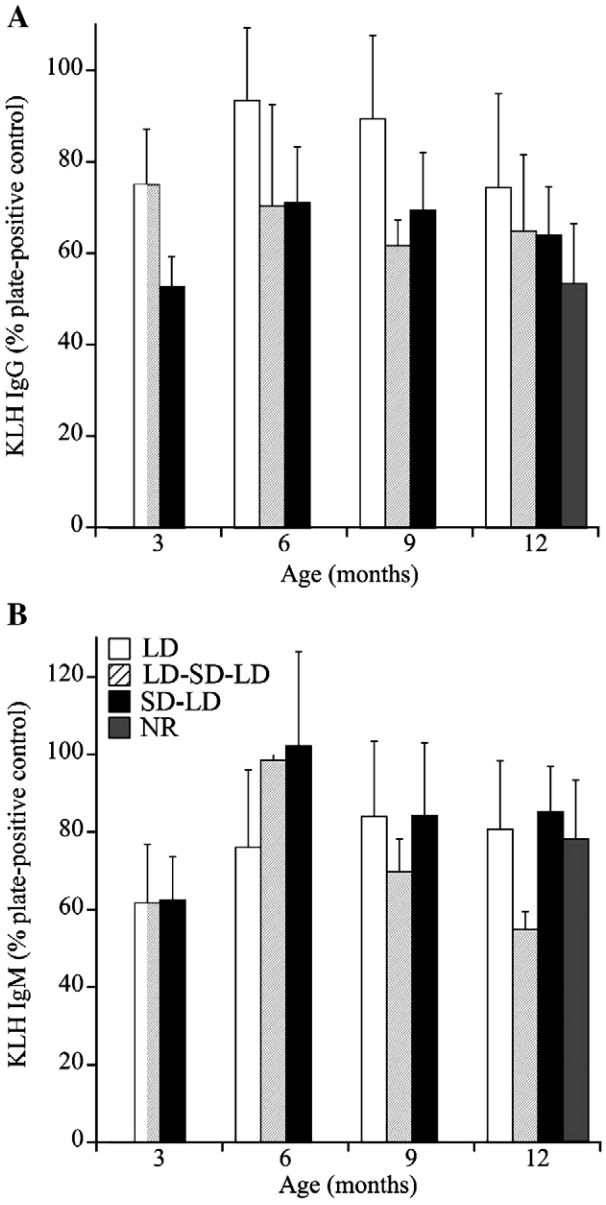


Fig. 4. Mean serum anti-KLH IgG (A) and IgM (B) levels, expressed as a percent of the plate-positive control (+SEM), in female hamsters just prior to when animals were euthanized at 3, 6, 9, or 12 months of age. Sample sizes were five to eight animals in all groups. None of the photoperiod-groups showed a significant change in KLH antibodies with age, nor were there any significant between-group differences at any age.

fewer primordial follicles than LD–SD–LD hamsters that demonstrated vaginal closure within 6 wk of transfer to SD and a sustained response to SD (vagina closed for 20 or more weeks).

Within the LD, LD–SD–LD, and SD–LD groups, the numbers of primary, secondary, and antral follicles declined significantly with advancing age, but within each of the four age classes no significant differences were found (Figs. 2–F).

Note that follicle counts for 6-mo-olds should be interpreted with caution, as sample sizes were smaller ($n=4$ to 5) than for the other age classes ($n=5$ to 8) because of suboptimal histology. Additionally, all of the SD–LD females representing this age group happened to have a patent vagina before reaching 6 months of age, which means they had become refractory to the inhibitory effects of SD. The significance of this will be discussed later, as the robustness of the SD response appeared to impact the size of the follicular pool at older ages.

Serum anti-Müllerian hormone

Within the LD and SD–LD groups, serum AMH concentration varied significantly across age classes, but the patterns of variation were different. Serum AMH concentration declined significantly by 9 months of age in LD females, whereas AMH concentration in SD–LD females was not significantly changed until 12 months of age. For LD–SD–LD females, age-associated changes in serum AMH concentration approached significance ($p=0.09$), and the pattern suggests a slower decline as compared to the LD group (Fig. 3). Within each of the four age classes significant differences in serum AMH concentration were noted between photoperiod groups at 6 months of age and the values at 9 months of age approached significance ($p=0.07$).

Because serum AMH levels appear to be affected by differences in prevailing photoperiod (Kabithe and Place, 2008), exploring the relationship of serum AMH concentration to ovarian follicle counts was limited to 12 months of age. This was the only sampling time when all hamsters in all groups were held in the same photoperiod, i.e., LD (16 L; see Table 1). The correlation of serum AMH concentration to the number of primordial follicles was modest, but significant ($r^2=0.17$, $p=0.04$). The correlation of AMH and follicle number was more substantial ($r^2=0.41$, $p=0.0004$) when the analysis was limited to the classes of follicles that represent the principal sources of AMH (primary + secondary follicles).

Fig. 2. (A) Mean number (+SEM) of primordial follicles counted in every 10th section of the right ovary in female hamsters at 3, 6, 9, or 12 months of age. (B) Individual primordial follicle counts for the three photoperiod groups at 9 months of age, and (C) for the four groups at 12 months of age. (D–F) Mean number (+SEM) of primary, secondary, and antral follicles counted in every 10th section of the right ovary in female hamsters at 3, 6, 9, or 12 months of age. Sample sizes were five to eight animals in all groups, except at 6 months ($n=4$ to 5). Each of the photoperiod-groups (LD, LD–SD–LD, and SD–LD) showed a significant decline ($p<0.05$) in the numbers of primordial, primary, secondary, and antral follicles with age ($p<0.05$), but no between-group differences were detected at any age. Note: None of the 12-mo-old LD females had any antral follicles, as indicated by the 0.

Anti-KLH IgG and IgM

Overall, neither anti-KLH IgG nor IgM demonstrated a significant decline with age in the LD group, therefore modulation of age-associated changes by photoperiodic history could not be detected (Figs. 4A and B). Generally, anti-KLH IgG levels were higher in LD females than in age-matched photo-inhibited females (SD–LD at 3 months and LD–SD–LD at 9 months). No recognizable patterns related to age or photoperiod could be discerned from the anti-KLH IgM data.

Discussion

The present study provides evidence that the deceleration of reproductive aging by SD in Siberian hamsters may occur when females develop in SD or when females are raised in LD and transferred to SD as young adults. Thus, the previous findings of Place et al. (2004), whereby reproductive aging was decelerated in females raised in SD, cannot simply be attributed to a general shift in reproductive life history states to later ages. The present study supports previous findings that SD decelerate the attrition of ovarian primordial follicles (Place et al. 2004, Timonin et al. 2006). However, the effects of SD on reproductive aging were not absolute, as a response to SD that was delayed or of relatively short duration was insufficient to maintain a substantial number of ovarian primordial follicles at advanced ages. These results highlight the importance of expanding the categorization of photoperiodic animals beyond the standard descriptors of responder or nonresponder (Prendergast et al., 2001), because within the responder group there will be subsets of animals that manifest a robust response to SD whereas others may demonstrate a more modest response (Butler et al., 2007). The difference in outcomes (number of primordial follicles and serum AMH concentrations) between robust and modest SD responders may have been more pronounced had the duration of SD been extended beyond 6 months. Several of the robust responders had not become refractory to SD when they were transferred to LD, at which time they were photo-stimulated and vaginal patency followed shortly thereafter. Place et al. (2004) maintained female hamsters in SD through 8 months of age, and many animals remained photo-inhibited well past 6 months of age. Thus, if SD–LD and LD–SD–LD females from the present study had been held in SD for longer than 6 months' time, the robust responders may have carried a larger follicular reserve into advanced age.

Because reproductive and somatic aging are so thoroughly intertwined in life history theory (Williams, 1966; Stearns, 1992), a reasonable question to ask is whether females that are responsive to SD live longer than females held in LD or than SD nonresponders. Longevity studies are costly and time consuming, thus as an alternative we have searched for biomarkers that consistently deteriorate with age. Whereas studies of the IgG response to a KLH challenge in *Phodopus* have been informative with regard to seasonal variation in immune function (Bilbo et al., 2002; Prendergast et al., 2004; Demas and Sakaria 2005), we did not find a reduction in KLH-IgG across the age range studied (3 to 12 mo). Therefore, KLH-IgG did not prove to be a useful marker for assessing the potential for SD to modulate somatic aging. In a separate pilot study, we have also investigated the possibility of using the attrition of telomere length as a biomarker of somatic aging, but a lack of difference in telomere length in cohorts of 1-mo-old and 16-mo-old LD hamsters rendered this test uninformative (unpublished results). Other potential markers (e.g., double-strand DNA breaks, T-cell subsets) are under consideration, but ultimately longevity studies may be required to determine if photoperiod modulates somatic aging in Siberian hamsters and other photoperiodic species.

Owing to the effects of SD on reproductive aging, and the variability in the SD response, chronological age may be a poor predictor of reproductive potential, especially in older females. Serum AMH concentration may be a better predictor, and it is being used to assess the likelihood of success when subfertile women present to clinics for assisted reproductive technologies (Fauser et al., 2008). Serum AMH decreases with age in female mice and reflects the size of the primordial follicle pool (Kevenaar et al., 2006), and it may help to predict the age of menopause in women (van Disseldorp et al., 2008). Age-associated changes in serum AMH levels in Siberian hamsters are confounded by photoperiod, as the concentration in juvenile SD females was lower than in age-matched LD animals, even though ovarian AMH levels were 3-fold higher in SD than in LD at 10 wk of age (Kabithe and Place, 2008). Decreased blood flow to and from the photo-regressed ovary and the avascular nature of the AMH-expressing hypertrophied granulosa cells within SD ovaries has been postulated as possible mechanisms for the lower serum AMH concentration in juvenile SD females (Kabithe and Place, 2008), but this remains to be determined. The value of serum AMH concentration as a predictor of reproductive potential in hamsters is in progress, as we await new cohorts of LD, SD–LD, and LD–SD–LD females to reach a year's age for breeding trials, at which time we will determine if females with higher AMH levels are more likely to produce litters. Maternal behavior of 1-year-old dams will also be evaluated, because Place et al. (2004) found the body mass of individual pups at weaning was significantly less for older dams maintained in LD than for females raised in SD.

In conclusion, knowing photo-inhibition of reproductive physiology in adult hamsters can decelerate reproductive aging has important implications for understanding the life history strategy that individual females pursue as photoperiod decreases toward the end of the breeding season. If supplemental information, such as environmental temperature, modulates the response to decreasing day length – in terms of duration and the timing of onset and offset – then recent temperature trends associated with climate change could impact the capacity for photo-inhibition to decelerate reproductive aging and preserve fertility for future reproductive efforts. As evident in the present study, a relatively rapid and sustained response to SD is associated with the greatest preservation of the ovarian follicular reserve.

Female hamsters born at the extremes of the breeding season have an easy 'decision' with regard to their life history strategy — those born early in the year mature and reproduce quickly, whereas those born late in the year delay puberty and first reproductive effort until the following spring. However, some females will be caught in the middle, i.e., they are born near the summer solstice and may produce one or more litters before they confront shorter days and conditions less conducive to breeding. Should they persist in their reproductive effort and the associated sexual behaviors, or adopt an alternate strategy by transitioning to the SD phenotype, which has the potential to decelerate reproductive aging and thereby preserve reproductive potential until favorable breeding conditions have returned? The internal and external cues used to make that 'decision' have yet to be elucidated.

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