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Age of ovary determines remaining life expectancy in old ovariectomized mice

Shelley L. Cargill[1], **James R. Carey**[2], **Hans-Georg Müller**[3], and **Gary Anderson**[1]

1*Department of Animal Science, University of California, Davis, CA 95616, USA*

2*Department of Entomology, University of California, Davis, CA 95616; and Center for the Economics and Demography of Aging, University of California, Berkeley 94720, USA*

3*Department of Statistics, University of California, Davis, CA 95616, USA*

Abstract

Summary—We investigated the capacity of young ovaries, transplanted into old ovariectomized CBA mice, to improve remaining life expectancy of the hosts. Donor females were sexually mature 2-month-olds; recipients were prepubertally ovariectomized at 3 weeks and received transplants at 5, 8 or 11 months of age. Relative to ovariectomized control females, life expectancy at 11 months was increased by 60% in 11-month recipient females and by 40% relative to intact control females. Only 20% of the 11-month transplant females died in the 300-day period following ovarian transplantation, whereas nearly 65% of the ovariectomized control females died during this same period. The 11-month-old recipient females resumed oestrus and continued to cycle up to several months beyond the age of control female reproductive senescence. Across the three recipient age groups, transplantation of young ovaries increased life expectancy in proportion to the relative youth of the ovary. Our results relate to recent findings on the gonadal input upon aging in *Caenorhabditis elegans* and may suggest how the mammalian gonad, including that of humans, could regulate aging and determine longevity.

Keywords

mouse life table; mouse mortality; life span; ovary transplantation; gonadal signals

Introduction

Although it is widely known that aging in many species is inextricably linked to different aspects of reproduction (Partridge & Harvey, 1985; Bell & Koufopanou, 1986; Gosden, 1996), little is known about the direct effect of gonadal input on longevity in mammals. Many studies have examined the effects of ovarian manipulation on reproductive capacity (Aschheim, 1965; Felicio *et al.*, 1983; Mobbs *et al.*, 1984). However, the nature and impact of gonadal inputs on longevity are unclear because these experimental designs confounded age of ovary and age of individual. Here, to define the direct ovarian influence on longevity in mice, we prepubertally ovariectomized females and subsequently transplanted young ovaries into these females at older ages.

Four concepts and observations shape our experimental design. First, model species including medflies (Carey *et al.*, 1998a) and mice (Merry & Holehan, 1979) suggest that dietary restriction does not independently arrest reproduction and reduce mortality. Rather, reduced

Correspondence, James R. Carey, Department of Entomology, One Shields Ave., University of California, Davis, CA 95616, USA. Tel.: +1 530 752 6217; fax: +1 530 752 1537; e-mail: jrcarey@ucdavis.edu.

mortality produced by diet restriction is a consequence of the arrested reproduction produced by the diet restriction. This conclusion follows from studies with medflies and with mice in which females are switched from *ad libitum* to restricted diets and their reproductive capacity and post-reproductive mortality are restored to levels that are virtually identical to individuals maintained on *ad libitum* diets (Merry & Holehan, 1979; Carey *et al.*, 1998a). With the medfly, Müller *et al.* (2001) modelled this demographic outcome as a 'reproductive clock' in which the rate of reproductive decline is a good predictor of the speed of aging.

Second, in the nematode *Caenorhabditis elegans*, Kenyon and co-workers (Hsin & Kenyon, 1999; Arantes-Oliveira *et al.*, 2002) show that ablation of the full gonad does not affect life span but that selective ablation of the germ-line stem cells significantly increases longevity. These data suggest there is a balance between germ line tissue and gonadal somatic tissue signals that regulates life span. In mice we may then expect the nuances of reproduction, including the presence or absence of cycling and its intensity, to affect the relationship between reproduction, mortality and longevity.

Third, in medfly we find that mortality rate is a graded function of the physiological age of the ovary. Ovarian physiological status can have opposite effects on mortality – a physiologically young ovary can decrease mortality while a physiologically old ovary can increase mortality (Carey *et al.*, 1998a, 2002). Operationally, in response to access to a full (protein-rich) diet, female medflies experienced reduced mortality early in the 'reproductive' mode (i.e. physiologically young ovaries) but heightened mortality in the later stages of this mode (i.e. physiologically old ovaries). This observation suggests that the pattern of mortality in experimentally manipulated animals will depend on the age of their transplanted ovaries. In mice we predict that young ovaries will depress mortality but old ovaries will heighten mortality causing a precipitous drop in survival as the cohort reaches the most advanced ages.

Fourth, the age-effects of reproduction on mortality are strongest at older ages because young individuals are intrinsically robust and neither need nor can benefit from the protective effect provided by youthful ovaries. We see this in medflies when females are switched from a sugar to a sugar-plus-yeast diet as young or as old adults (Carey *et al.*, 1998a). Females fed sugar only have high mortality and exhibit arrested reproduction. When switched to sugar plus yeast as young females, mortality is modestly decreased while reproduction is increased, but when switched as 90-day-old females, mortality is strongly decreased relative to the increase in reproduction which is maintained.

An effective design therefore must include the following specific elements. (1) There should be no ovarian input prior to ovary transplantation. When properly controlled in this way, any change in mortality when ovaries are transplanted can be attributed to the presence of these ovaries and not to input from ovaries carried through from younger ages. In our study we therefore ovariectomized (OX) the treatment and control mice prior to sexual maturation (i.e. in prepubescent animals at 3 weeks of age). (2) Treatments should be initiated at several ages. From the age of 11 months, females that received transplanted young ovaries at 5, 8 or 11 months continue to age with ovaries that are, respectively, 8, 5 and 2 months old. The design initiates ovarian transplants across the full range of reproductive capacity (early, peak and declining, respectively) and tests how ovarian age affects life expectancy of similarly aged females. (3) Controls are required with permanently ovariectomized individuals accompanied by sham ovarian transplant surgery (OX control) and with intact individuals (IT control). (4) Ovaries should be transplanted into the site of origin, the ovarian bursa. Since signals between germ line tissue and somatic tissue may influence aging, it is crucial to locate the transplant within the physiologically appropriate tissue. Using these four design elements, we were able to manipulate specifically the reproductive system of the mouse, allowing us to examine directly the influence of reproduction on aging.

Results

Ovarian transplantation at 11 months in mice with prepubertal ovariectomy significantly increased remaining life expectancy by 60% (136.9 days) relative to OX control (Table 1). In contrast, relative to OX control at 11 months, the remaining life span was only marginally increased in females that received young ovaries at 5 or 8 months of age (Table 1). As a point of reference, CBA females are normally post-reproductive at 11 months (Fernandes *et al.*, 1973), and young ovaries increase life span when transplanted to females at this age.

Survivorship normalized to 100% at age 11 months demonstrates important differences among the treatment and control cohorts (Fig. 1). In the 11-month transplant cohort the first 50% of all deaths occurred over 430 days while the remaining 50% of deaths occurred much more rapidly across the following 100 days. In contrast, among OX and IT control cohorts the median age of death was at 316 and 250 days, respectively. Only 20% (four of 20 mice) of the 11-month transplant cohort died during the 300-day period following ovarian transplantation, despite having been subjected twice to major surgery. In contrast, 64% (14 of 22 mice) of the OX control cohort died during the same period. This late acceleration in mortality rate in the 11-month transplant cohort suggests an interaction between the reproductive system and organismal-level aging. Transplantation of young ovaries at 11 months appears to postpone aging by shifting the survival distribution to the right as well as by changing its slope.

We estimated the impact of ovarian transplantation on remaining life expectancy at 11 months with a linear individual-based model including females of the IT control and the three transplant cohorts (Fig. 2). Donor mice were aged 2 months, and recipient mice were aged 5, 8 and 11 months. Eleven-month-old females therefore carried ovaries that were, respectively, 8, 5 and 2 months old. The IT control, at 11 months, carried 11-month-old ovaries. Remaining life span from age 11 months, R_i, was modelled as $R_i = b_0 + b_1 * OVAGE_i + \varepsilon$ with parameters estimated by least squares. With OVAGE = 2, 8, 5 and 11 months, $b_0 = 364.4$ and $b_1 = -10.2$ ($F = 5.72$, $P < 0.02$). Excluding the IT females, which did not undergo surgical manipulation, $b_0 = 402.3$ and $b_1 = -20.2$ ($F = 7.72$, $P < 0.01$). Thus, remaining life expectancy at 11 months decreased by at least 10.8 days for each additional month of ovary age. Beyond a simple qualitative effect of presence or absence of a transplanted ovary, we see that remaining life span is proportional to the relative youth of the ovary.

Since the 5- and 8-month transplant cohorts had only small gains in remaining life expectancy relative to OX and IT controls, it seems that the protective effect of the new ovary for these cohorts could be masked because of their own somatic youth. The protective effects of the young ovaries and the intrinsic youthfulness of the mice were redundant. This interpretation is consistent with the oestrous cycling behaviour of transplanted and control females. Event history charts (Carey *et al.*, 1998b) demonstrate how ovarian transplantation postpones reproductive aging (Fig. 3). The duration of oestrous cyclicity after 11 months was markedly extended in the 11-month transplant females relative to all other cohorts. In contrast, young ovaries transplanted to young females make relatively little contribution to life expectancy or to reproductive schedule.

Discussion

Life span can be extended in mammals by caloric restriction (Weindruch & Walford, 1998) and by mutations of the endocrine system (Mobbs *et al.*, 1984). These manipulations change many integrated systems, including reproduction. Here, using transplantation we varied ovary age independently of chronological age and demonstrate that reproductive state is a primary determinant of mammalian life span. Transplantation of a young ovary into a female at 11 months of age extends life expectancy of the host by 60%. Furthermore, the younger the ovary

when a mouse reached 11 months (i.e. the more recent the transplantation), the greater the benefit conferred in terms of life expectancy at that age. Females receiving ovaries at relatively young ages show little added benefit relative to controls, either for life span or for future reproduction. Youth, as it were, is wasted on the young. Remarkably, 11-month-old recipients of 2-month-old ovaries restored oestrous cycling, sometimes for 200 days beyond the age of last reproduction in control females. The young ovary retards both the somatic and the reproductive aging of the host.

A key role for gonadal control of aging has been recently considered in developmental studies of *C. elegans*. Patel *et al.* (2002) suggested that variation in germ-line stem cell signalling contributes to body size and life history diversity in nematodes. Hsin & Kenyon (1999) postulated that the gonad of *C. elegans* produces two counteracting signals – one produced by the germ-line cells which shortens life span (Arantes-Oliveira *et al.*, 2002) and the other produced by the somatic gonad, lengthening life span. Thus, ablation of the germ-line cells extends life span, while ablation of the entire gonad removes both signal sources and does not alter longevity. In our study, females aged without a gonad until they were 11 months old, at which time they received a young gonad. If the somatic function of the young gonad was retained but the germ-line-derived activity was reduced, these ovaries could signal for slow-aging in the somatic host.

Our design for ovarian transplantation may have produced these precise conditions because follicles are lost due to ischaemia during ovarian transplantation in rodents. Thus, the number of gametes is reduced while the quantity of somatic gonadal tissue, which is less sensitive to the decreased blood supply, remains relatively constant. We saw that oestrous cycles were restored in the 11-month-old transplants but not to the level of young IT controls. If the oestrous patterns accurately reflect underlying germ-line-dependent endocrine activity of these ovaries, these data suggest that the transplanted ovary may release strong signals from the young somatic gonad and relatively weak ones from an attenuated germ-line. Thus, the net signal strength favours a reduction in aging rate, resulting in an increase in longevity.

Young ovaries have a protective effect on the soma, especially when transplanted into older mice. Young ovaries may have protective effects in young and middle-aged mice but this may be redundant when combined with the intrinsic hardiness of the younger adult's somatic youth. Endocrine signals across somatic and germ-line tissues may represent an adaptation of the germ-line to ensure its continuity through regulation of somatic endurance. The accumulating evidence from previous studies of the influence of reproduction on longevity coupled with the current findings suggests that there exists a conserved system to regulate survival mechanism across broad groups of species (Ideker *et al.*, 2001).

Conclusions

With a few recent exceptions (see Tatar, 2002, for a review) little attention has been directed in gerontology to determine the role of reproduction in senescence and longevity. The research results presented here show that transplanted young ovaries received by hosts at older ages can extend the life span. These data are the first to establish that ovarian function plays a direct role in how mammals age. Our findings set the stage for future research to determine how transplanted young ovaries may extend life span in non-ovariectomized older mice, to determine whether second transplants of young ovaries can further extend life span at older ages, and to understand basic cellular and molecular mechanisms underlying ovarian regulation of longevity. Our results have important implications for understanding human aging because they interweave with many cross-threads of research in model organisms and systems, establishing the primacy of gonadal input in mammalian aging and longevity. The results also underscore the need to re-visit the relationship of longevity to the indicators of reproductive

aging in women including the age of onset of menopause (Snowdon *et al.*, 1989), child-bearing ability at later ages (Perls *et al.*, 1997) and childlessness (Westendorp & Kirkwood, 1998; Müller *et al.*, 2002).

Experimental procedures

Animals

Mice of CBA/J strain were housed under controlled conditions of temperature (21 ± 2 °C), humidity (minimum 50%) and lighting (14L : 10D) according to the American Association for the Accreditation of Laboratory Animal Care guidelines for animal care. Animals received feed and water *ad libitum*.

Surgical procedures

Prepubertal female mice were ovariectomized (OX) at 21–22 days. Bilateral ovariectomies were performed as previously described (Cargill *et al.*, 1999) except that one sterile 1-mm-diameter glass bead was inserted into each empty ovarian bursa to keep it open for future ovarian transplantation. After surgery, each female was housed individually in a 26 × 17 × 13-cm shoe-box cage in a specific-pathogen-free colony monitored regularly for the duration of experiments. At 5, 8 or 11 months, bilateral ovarian transplantation surgery was performed as previously described (Cargill *et al.*, 1999) with the exception that the glass bead was first removed and an ovary from a 2-month-old donor female of the same strain was placed into each ovarian bursa. The OX control cohort underwent sham ovarian transplant surgery at 8 months in which the glass bead was removed, but no ovary was transplanted. The intact control cohort was housed under the same conditions. As a result of ovariectomy, treated animals were not exposed to ovarian input until ovarian transplantation at 5, 8, or 11 month (n = 22, 27, 20, respectively). Control animals were either non-surgical, intact (IT) (abbreviation defined previously) (n = 28) or were ovariectomized as before, followed by sham surgery at 8 months, OX (n = 25). Reproductive data in the form of vaginal cytology were collected daily pre- and post-transplantation to ensure complete removal of the ovarian tissue and success of the ovarian transplantation procedure.

Statistical analysis

Differences in the distribution of duration of cycling post 11 months were established with a log-rank test for the four groups with an ovary present at 11 months, since there was censoring due to the death of mice. The test yielded $\chi^2 = 49.1$ with 3 degrees of freedom (d.f.), resulting in $P < 0.0001$, thus providing strong evidence for differences in cycle duration. For assessment of time-to-death, graphical analysis showed that an assumption of proportional hazards was invalid and therefore analysis was based on least-squares regression analysis for time-to-death as response, which was facilitated by the fact that time-to-death was observed for all subjects without censoring. Residuals did not deviate from the assumption of normality. Quadratic terms were added to further assess goodness-of-fit but did not improve fit of the regression model with linear predictors. An additional model was considered to compare the intact control group to all ovariectomized groups where OVNEW is 1 for new ovary transplanted at 11 months and OVNEW is 0 for IT control. Remaining lifetime $R_i = b_0 + b_1 * OVNEW_i$ is estimated with b_0 and b_1 as 232 and 136.9, respectively ($P < 0.004$); remaining life expectancy was increased by 136.9 days due to transplantation of a young ovary at 11 months compared with the IT control group.

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References

Arantes-Oliveira N, Apfeld J, Dillin A, Kenyon C. Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. Science 2002;295:502–505. [PubMed: 11799246]

Aschheim P. Resultats fournis par la greffe heterochrone des ova-rie dans l'etude de la regulation hypothalamus-hypophyso-ovarienne de la ratte senile. Gerontologia 1965;10:65–75. [PubMed: 14312531]

Bell, G.; Koufopanou, V. The cost of reproduction. In: Dawkins, R.; Ridley, M., editors. Oxford Surveys in Evolutionary Biology. Oxford: Oxford University Press; 1986. p. 83-131.

Carey JR, Liedo P, Harshman L, Liu X, Müller H-G, Partridge L, Wang J-L. Food pulses increase longevity and induce cyclical egg production in Mediterranean fruit flies. Funct. Ecol 2002;16:313–325.

Carey JR, Liedo P, Müller H-G, Wang J-L, Vaupel JW. Dual modes of aging in Mediterranean fruit fly females. Science 1998a;281:996–998. [PubMed: 9703516]

Carey JR, Liedo P, Müller H-G, Wang J-L, Vaupel JW. A simple graphical technique for displaying individual fertility data and cohort survival: case study of 1000 Mediterranean fruit fly females. Funct. Ecol 1998b;12:359–363.

Cargill SL, Medrano JF, Anderson GB. Infertility in a line of mice with the *high growth (hg)* locus is due to luteal insufficiency resulting from disruption at the hypothalamic-pituitary axis. Biol. Reprod 1999;61:283–287. [PubMed: 10377060]

Felicio LS, Nelson JF, Gosden RG, Finch CE. Restoration of ovulatory cycles by young ovarian grafts in aging mice: potentiation by long-term ovariectomy decreases with age. Proc. Natl Acad. Sci 1983;80:6076–6080. [PubMed: 6577467]

Fernandes G, Yunis EJ, Good RA. Reproductive deficiency of NZB male mice. Possibility of a viral basis. Lab. Invest 1973;29:278–281. [PubMed: 4744087]

Gosden, R. Cheating Time: Science, Sex and Aging. New York: W.H. Freeman; 1996.

Hsin H, Kenyon C. Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature 1999;399:362–366. [PubMed: 10360574]

Ideker T, Galitski T, Hood L. A new approach to decoding life: systems biology. Annu. Rev. Genomics Hum. Genet 2001;2:343–372. [PubMed: 11701654]

Merry BJ, Holehan AM. Onset of puberty and duration of fertility in rats fed a restricted diet. J. Reprod. Fertil 1979;57:253–259. [PubMed: 513013]

Mobbs CV, Gee DM, Finch CE. Reproductive senescence in female C57BL/6J mice: ovarian impairments and neuroendocrine impairments that are partially reversible and delayable by ovariectomy. Endocrinology 1984;115:1653–1662. [PubMed: 6541567]

Müller HG, Carey JR, Wu D, Vaupel JW. Reproductive potential determines longevity of female Mediterranean fruitflies. Proc. Roy. Soc. London B 2001;268:445–450.

Müller H-G, Chiou J-M, Carey JR, Wang J-L. Fertility and lifespan: Late children enhance female longevity. J. Gerontol. Biol. Sci 2002;57A:B202–B206.

Partridge L, Harvey PH. Cost of reproduction. Evol. Biol 1985;316:20.

Patel MN, Knight CG, Karageorgi C, Leroi AM. Evolution of germ-line signals that regulate growth and aging in nematodes. Proc. Natl Acad. Sci 2002;99:769–774. [PubMed: 11805331]

Perls TT, Alpert L, Fretts RC. Middle-aged mothers live longer. Nature 1997;389:133. [PubMed: 9296486]

Snowdon DA, Kane RL, Beeson WL, Burke GL, Sprafka JM, Potter J, Iso H, Jacobs DR, Phillips RL. Is early natural menopause a biologic marker of health and aging? Am. J. Public Health 1989;79:709–714. [PubMed: 2729468]

Tatar M. Germ-line stem cells call the shots. Trends Ecol. Evol 2002;17:297–298.

Weindruch, R.; Walford, RL. The Retardation of Aging and Disease by Dietary Restriction. Springfield, IL: Charles C Thomas; 1998.

Westendorp RGJ, Kirkwood TBL. Human longevity at the cost of reproductive success. Nature 1998;396:743–746. [PubMed: 9874369]

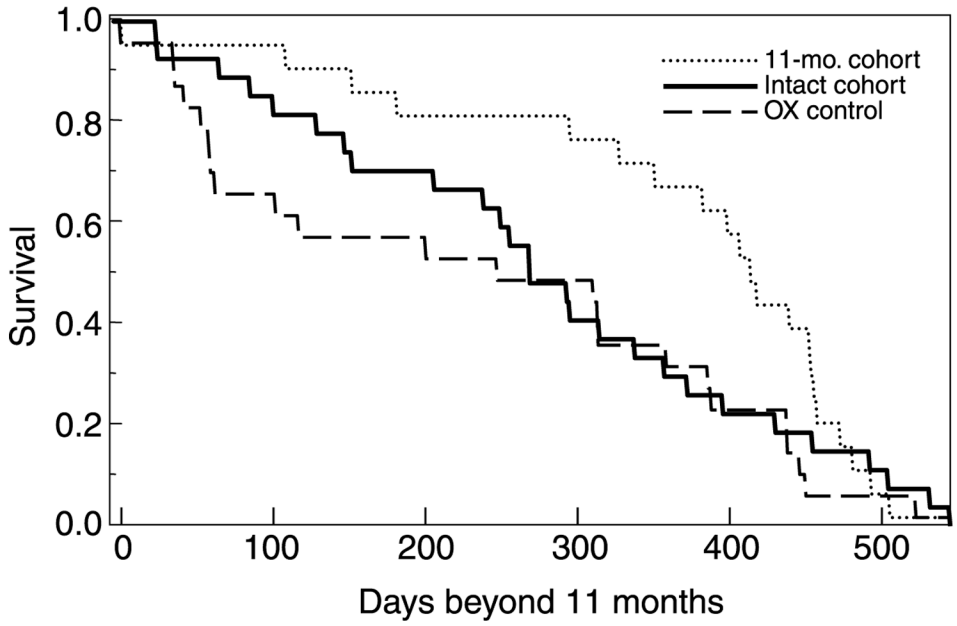

Fig 1.

Cohort survival for the IT and OX control and 11-month transplant cohorts normalized to 100% at 11 months, where 27, 23 and 21 mice were alive in IT control, OX control and 11-month treatment cohorts, respectively.

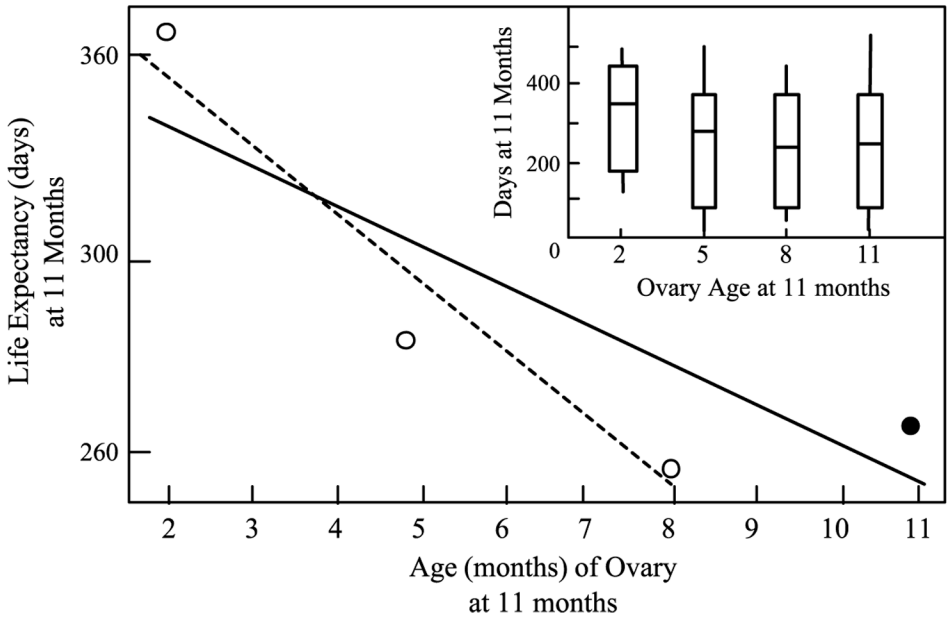

Fig 2.

Regression of remaining life expectancy at 11 months as a function of ovary age at 11 months. Regressions based on individual life span with groups treated as repeated measures. Solid line includes intact control (open and filled circles) and dashed line excludes intact control (open circles only). Inset: Box plots for mouse deaths (days) beyond 11 months. The high–low extremes of the rectangles indicate values for the mid-50% of the distribution, the horizontal lines indicate the means, and the vertical lines indicate the ranges.

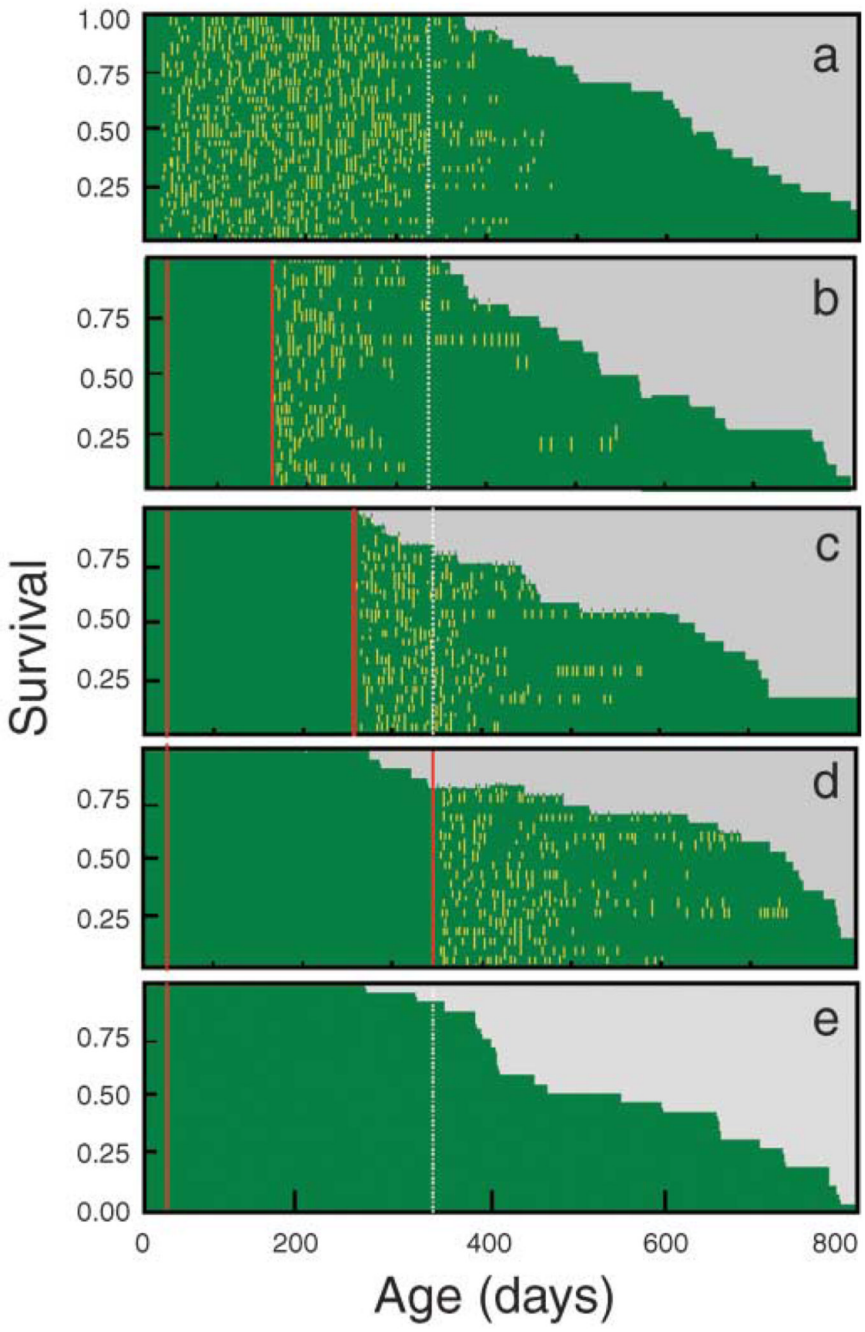

Fig 3.

Event history graphs (Carey *et al.*, 1998b) of mouse survival and ovarian cycle data for control and treatment cohorts. Each individual within a panel is represented by a horizontal 'line' proportional to her life span and rank-ordered from shortest- (top) to longest-lived (bottom). The left-most red vertical lines indicate the age of ovariectomy (3 weeks), the right-most vertical red lines indicate the ages of transplantation for the three treatment cohorts, and the vertical yellow ticks indicate the start of new cycles for individual mice. (a) Intact (IT) control; (b) 5-month transplant; (c) 8-month transplant; (d) 11-month transplant; (e) permanently ovariectomized (OX) control. The vertical dashed white lines at 11 months in a, b and c are included as a reference point for cycling activity in these cohorts at older ages relative to the

11-month cohort. Significantly longer cycling for the 8-month OX group ($P < 0.001$) and the 11-month OX group ($P < 0.000001$) corroborates the graphical evidence. Note the relatively flat survival trajectory in the 8- and 11-month transplants (panels c & d) from 330 days (11 months) to about 750 days relative to both the IT (a) and OVX (e) controls.

Table 1

Remaining life expectancy for mice in five cohorts of female CBA mice. Treatments: prepubertal ovariectomy with receipt of young transplant ovary at 5, 8 and 11 months; intact (IT) control; prepubertal ovariectomy without transplant (OX control), and long-term ovariectomy (OX) control cohorts. Value in parentheses gives the number alive at the specific age. Life expectancy of each transplant cohort was compared at 11 months to both the IT and the OX controls and each contrast was significant ($P < 0.05$)

Treatment	Remaining Life Expectancy (days) at:			
	ovariectomy*	5 months	8 months	11 months
IT Control	598.5 (28)	444.5 (28)	346.5 (28)	**265.6** (27)
OX Control	539.9 (25)	385.9 (25)	300.2 (25)	**230.5** (23)
5-mo. transplant	575.2 (22)	421.2 (22)	323.2 (22)	**245.8** (21)
8-mo. transplant	549.5 (27)	395.5 (27)	297.5 (26)	**285.9** (21)
11-mo. transplant	627.2 (25)	473.2 (25)	375.2 (25)	**367.3** (21)

* Ovariectomy performed at 3 weeks

Values in bold type highlight life expectancy in control and treatment mice at 11 mo