Published in final edited form as:

Ann N Y Acad Sci. 2010 August ; 1204: 149–155. doi:10.1111/j.1749-6632.2010.05522.x.

Reproductive Aging in Invertebrate Genetic Models

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Abstract

The invertebrate genetic systems of *C. elegans* and *D. melanogaster* are emerging models to understand the underlying mechanisms of reproductive aging and the relationship between reproduction and lifespan. Both animals show progressive decline in egg production beginning at early middle age, caused in part by reduction in germline stem cell proliferation as well as in survival of developing eggs. Molecular genetic analysis reveals that insulin and TGF- β signaling are regulators of germline stem cell maintenance and proliferation during aging. Furthermore, the lifespan of both *C. elegans* and *D. melanogaster* appears to be regulated by signaling that depends on the presence of germline stem cells in the adult gonad. These invertebrate models provide powerful tools to dissect conserved causes of reproductive aging.

Keywords

C. elegans; *D. melanogaster*; stem cells; reproductive aging; insulin; TGF- β

Reproductive senescence is the process whereby adults have progressively reduced fertility and fecundability with advancing age, beginning in human females sometime in the fourth decade and progresses until menopause approximately two decades later.¹ The way we understand such human reproductive senescence is shaped by the fact that all oocytes are produced during prenatal development. Human females are born with a finite pool of arrested primordial follicles. Reproductive senescence may be inevitable because the size of this pool may only decrease with age, and because the quality of the quiescent eggs diminishes with time. Reproductive senescence, however, is not just a phenomenon of mammals. In the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* fecundity declines beginning at young to middle ages until females cease reproduction while often remaining viable. Yet, in contrast to mammals, primary oocytes are produced from stem or progenitor cells within the mature adult in both the worm and the fly. Given this difference in timing of primary egg production, are there any common threads between the processes of reproductive senescence of mammals and of these invertebrate models? Perhaps there is – these invertebrates regulate aspects of their life history, including development, reproduction and lifespan, with Transforming Growth Factor *beta* (TGF- β) and insulin/IGF hormones. Animals may have common, ancestral signaling networks to regulate the propensity for reproductive decline, and to control the relationship between reproduction and lifespan.

Nematode reproduction and its senescence

The nematode *C. elegans* develops from a zygote to a mature adult through four larval stages. Adults are typically hermaphroditic; males can develop but are rare. Cells that will

produce the somatic gonad and the germ lineage of hermaphrodites first arise at early embryogenesis.² Cells of the Z1/Z4 lineages go on to produce the somatic tissues of ovary and testes, while cells of the Z2/Z3 lineage produce germline progenitors. Germline progenitor cells make a finite number of sperm during the fourth larval stage of development. At the transition to adulthood, these germline progenitor cells switch to become the continually dividing source for oocytes.

C. elegans biologists work with standard inbred stocks. A common wildtype strain is N2. Eggs from this strain hatch and develop to adults in about 3 days, unless they pass into an alternative resistant, non-feeding dauer state that lasts up to many weeks and then transition back to larval stage 4 before molting to an adult. Adults from both developmental pathways begin to reproduce immediately, fertilizing newly generated eggs with their own sperm. Egg laying peaks in adults at ages 2 to 3 days and thereafter declines rapidly. Self-fertilizing hermaphrodites lay about 250 to 300 fertilized eggs by age 4 to 5 days old. Unfertilized eggs are also oviposited, with a peak at about day 2 but continuing to age 8 or 9 days.³

These observed patterns of reproductive decline may arise simply because hermaphrodites are limited by their fixed store of sperm, much as human female reproductive span may be limited in the number of primary follicles. For *C. elegans* this hypothesis can be addressed experimentally. Mated hermaphrodites show that reproduction is not sperm limited.⁴ In these adults total reproduction increases to ~430 progeny but egg laying still begins to decline at about adult day 2 and ends at age 8 to 9 days. A similar pattern occurs in mutants of the F-box-containing protein encoded by *fog-2*, which are self-sterile and must mate to lay their eggs. Importantly, *fog-2* mutant adults mated at late ages produced few early progeny but still displayed a typical pattern of reproductive decline. Not only is reproductive senescence independent of sperm availability, it is not accelerated by early reproduction or caused simply by the metabolic demands of reproduction.

Besides reproductive aging, *C. elegans* is a key model to study genetic factors regulating lifespan. While these studies largely focus on longevity assurance, some data address the interaction between genes conferring longevity and their impact on reproduction. Insulin/IGF signaling, in particular, has been dissected as a regulator of longevity. Mutants of the insulin-receptor encoded by *daf-2* increase longevity about two fold.⁵ In self-fertilizing hermaphrodites several but not all *daf-2* alleles produce some progeny at late ages and delay age-associated change in adult gonad morphology.^{6, 7} The effect of *daf-2* on the reproductive schedule of mated hermaphrodites has been described only recently.⁴ While the *daf-2(e1370)* allele retards reproductive aging, other alleles of *daf-2* extend longevity without affecting the pattern of reproductive decline. These data suggest that the age-distribution of reproductive effort and the timing of reproductive senescence are not inexorably linked to lifespan. For the most part, genes extending the lifespan of *C. elegans* tend to do so by increasing post-reproductive life expectancy.⁴

Perhaps the most interesting feature of *C. elegans* reproduction in the context of aging is the way in which it can modulate lifespan. The original study showing extended longevity in mutants of *daf-2* included an interesting control – sterile worms.⁵ To rule out an explanation where *daf-2* increased lifespan simply because it reduced fecundity, a lifespan trial with wildtype animals was conducted with sterilized by laser ablation of the gonad. Survival in the sterile worms was indistinguishable from that of the fertile worms. Thus, the increase in longevity caused by *daf-2* could not be simply due to some effect on reproduction. But while useful for how we interpret *daf-2*, these data opened a paradox that was largely overlooked at the time. Contrary to expectations based on decades of empirical data in many animals,⁸ there was no apparent trade-off between reproduction and lifespan; eliminating the ‘cost of reproduction’ did not increase longevity.

Fortunately, this paradox was not lost upon the authors. In a subsequent study worms were made sterile by laser ablation of the gonad and germline precursor cells.¹¹ Ablation of Z1 and Z4 caused loss of the full gonad and these sterile worms had normal lifespan. Ablation of Z2 and Z3, however, only eliminated germline precursors, leaving adults with empty somatic gonads. Remarkably, these adults were long-lived. Thus, sterility does not modulate aging but loss of the germ-line increases lifespan. To interpret these data, Kenyon suggests that an active germline produces a pro-aging endocrine signal while the somatic gonad produces a longevity-assurance signal. Loss of both signals results in no net change in lifespan but loss of the negative germline signal alone permits the longevity assurance signal from the somatic gonad to increase lifespan.

We might understand reproductive senescence, including the process in mammals, if we can identify the somatic and germline longevity regulatory signals proposed to occur in *C. elegans*. The relevant literature is still very young. Initially, Hsin and Kenyon¹¹ established that the forkhead transcription factor *daf-16* was required for germline ablation to increase lifespan. DAF-16 is modulated by signals from DAF-2, the *C. elegans* insulin-like receptor. When DAF-2 is activated by insulin like peptides, a signal is transduced through the cell to keep DAF-16 in the cytoplasm, thereby preventing this transcription factor from inducing genes that slow aging. Thus, while *daf-2* mutation is argued to extend lifespan independently of its effects on the germline, the DAF-16 transcription factor must be involved in the way the germline modulates lifespan.

A second endocrine system that integrates reproduction and aging involves *daf-12*. DAF-12 is a nuclear hormone receptor that binds dafachronic acid, a bile acid-like steroid.⁹ *daf-12* was originally identified by its mutant effects on dauer formation and was subsequently described to influence metabolism, developmental timing and longevity.¹⁰ In unfavorable environments, the insulin/IGF and TGF- β signals are weak, and unliganded DAF-12 interacts with a corepressor to promote transcriptional programs favoring dauer and longevity.¹⁰ DAF-12 also plays a role in the way the germline modulates longevity since the extended lifespan of germline deficient adults requires wildtype *daf-12*.¹¹

Despite the power of these insights, neither insulin/IGF signaling nor *daf-12* yet describes how germ cells communicate with the soma to control aging, or reciprocally how reproductive senescence contribute to somatic aging process. But at this early stage we might suggest a place to look. As noted above, nematodes possess Transforming Growth Factor (TGF- β) signaling pathways. Although typically studied in the contexts of dauer formation and growth, TGF- β was recently shown to be a regulator of *C. elegans* adult lifespan.¹¹ This is important because the TGF- β superfamily includes the reproductively important mammalian hormones activin and inhibin.

The TGF- β pathway of *C. elegans* signals through two branches, the dauer branch and the so-called small/male abdomen branch.¹² In the dauer branch, a TGF- β like hormone encoded by *daf-7* activates a TGF Type I/Type II receptor complex encoded by *daf-1* and *daf-4*.¹³ This receptor then phosphorylates SMADs (homologs of both the drosophila protein, mothers against decapentaplegic (MAD) and the *C. elegans* protein SMA), which are proteins that repress TGF- β activated transcription factors that otherwise promote dauer formation. The small/male abdomen branch uses a different hormone as its ligand, the Dpp/BMP-like (DBL-1). Through a receptor with units encoded by *daf-4* and *sma-6*, DBL-1 leads to phosphorylation of a different set of Smads that help induce genes essential for cell growth and the development of male traits. Early studies of *C. elegans* TGF- β found no specific effect on lifespan even though TGF- β controls dauer, and this dauer regulation and lifespan are linked through shared insulin signaling.⁶ Reappraisal by Shaw solved this problem.¹¹ TGF- β mutants cause embryos to prematurely hatch within the mother so that the

ability of some mutants to slow aging was obscured by the high age-independent matricide. To solve this problem Shaw chemically inhibited egg development. In this condition TGF- β pathway mutants of the dauer formation branch increase longevity up to two-fold, and in an insulin-signal dependent manner.

It is too early to yet understand how these *C. elegans* genes slow aging or whether they also affect reproductive senescence but parallels to the mammalian TGF- β family suggest that solving these questions is just a matter of time. *daf-4* is homologous to the mammalian Activin-type IIB Receptor (ActRIIB).¹⁴ ActRIIB mutant mice have reduced follicle stimulating hormone (FSH), and perhaps as a consequence, delayed fertility, follicular atresia and reduced number of corpora lutea.¹⁵ In mammals, activin binds to ActRII, which then makes a functional complex with Receptor Type I. Activin ligands themselves are dimers made of activin β -subunits. Inhibins are also dimers, made from the inhibin α -subunit with one activin β -subunit. Inhibins interact with the ActRIIB but do not recruit Receptor Type I, thus competitively inhibiting the potential effects of activin dependent signaling.¹⁶ Inhibin-B, in particular, is associated with human reproductive aging since it is produced by activated follicles.^{17, 18} As the number of recruited follicles declines with age, the pituitary sees less inhibin-B. Activin is now unopposed, and FSH is produced at high levels characteristic of perimenopause and menopause. If *C. elegans* modulates their ovarian axis with TGF- β feedback signaling in a way similar to mammals, we might predict there will be a decline in some inhibin-like subunit with age and a correlated increase in gonadotropic hormone, perhaps one of the worm's 38 insulin-like peptides.¹⁹

Drosophila reproduction and its senescence

Both male and female *Drosophila melanogaster* produce gametes as adults from populations of stem cells within their gonads. Here we focus on female reproductive aging where output is easy to quantify in terms of egg production. Because male reproductive success is difficult to measure, much less is known about male reproductive aging although recent elegant work has documented changes in fertility, sperm production and stem-cell functions with age.^{20, 21, 22}

Females eclose as adults with a pair of ovaries that already produce early stage egg chambers.²³ Each ovary consists of ~10–20 ovarioles. At the terminal end of each ovariole is the germarium that contains a stem cell niche for germline stem cells.²⁴ Eggs chambers are formed in the germarium and progressively develop as they transit down the ovariole until they are fertilized at the posterior end of the common oviduct and then deposited. A typical germarium contains two or three germline stem cells (GSC). As these divide, one daughter cell remains as a GSC while the other differentiates into a cystoblast that subsequently undergoes four mitotic divisions to produce an interconnected set of 15 nurse cells with one oocyte. Follicle cells derived from somatic stem cells of the germarium encapsulate the germline-cyst cells to form an egg chamber.

Age-specific egg production is qualitatively similar among laboratory strains of *D. melanogaster*, although patterns vary somewhat in quantitative detail.^{25–28} Egg production for females maintained on a normal diet is very low in newly eclosed females, peaks at about age 5 to 10 d where 20 to 50 eggs are laid per day, and declines thereafter until about 30 to 50 days old. There are few data on post-reproductive life expectancy; unpublished observations from our group found old females to live about 2–3 days after last egg although some environmental manipulations could dramatically increase this duration. Reproductive senescence is also apparent at the level of egg quality.^{25, 26} Nearly all eggs hatch when laid by young females, but this rate progressively declines to about 50% in females 30 days old. Likewise, egg morphological defects increase with female age. Besides changes in external

markers of reproduction, there is a progressive shift in mitochondria DNA composition among eggs from aged females that carried two mitochondria DNA length variants within their GSC.²⁹

Several mechanisms may contribute to reproductive senescence in female *Drosophila*.³⁰ At a morphological scale, old females do not lay fewer eggs because they have reduced number of ovarioles.²⁶ However, within each ovariole the number of GSC is reduced with age (from ~ 2.2–2.6 at < 1 week to ~1.7–2.0 at > 5 weeks) but these differences are too small to account for the substantial drop in egg production.^{24, 26, 31, 32} In contrast, the rate of GSC proliferation is markedly reduced with age.^{26, 31} In ovarioles with two GSC, independent methods to measure the rate of germline stem cell divisions are reported to show 1.7-fold reduction from 3 to 25 days and 0.50-fold reduction thereafter. In the earlier period, these rates may account for the observed change in egg number but not so after 25 days old where egg production falls more than 6-fold. Finally, apoptosis of aging egg chambers are a major factor contributing to reproductive senescence. Apoptosis increases in the egg chambers of older females such that at age 40 days more than half of the eggs are marked for degradation.²⁶

The decrease in egg survival in aged *Drosophila* may parallel processes that occur in follicles of aging humans where cumulative damage from reactive oxygen molecules may impair the viability of developing antral follicles.³³ With *Drosophila* it is possible to experimentally explore this idea.³¹ Over-expression of antioxidant super oxide dismutase (SOD) in GSC or in the GSC niche increased the number of GSC in late age females. This study did not address, however, whether SOD over-expression rescued the apoptosis of developing egg chambers in old females. In an ancillary observation, expression of heat shock proteins in GSC reduces fertility,^{34, 35} and oxidative stress is known to induce the heat shock response.³⁶ Oxidative stress in aged follicles of mammals and in the newly produced egg chambers of aged *Drosophila* may contribute to reduced survival and quality of eggs during reproductive senescence.

Molecular analysis with *Drosophila* can also distinguish between effects on reproductive senescence from within the germline relative to those from without. These studies differentiate the roles played by the GSC, the ovarian stem cell niche and the systemic physiology external to the ovary. Developmental studies show that TGF- β from the stem cell niche is required for the early proliferation and maintenance of GSC.^{37, 38, 39} TGF- β ligands are secreted from niche cells and are received by the GSC through their ActRI/ActRII receptor.^{31, 37} Changes in such TGF- β signaling may contribute to fly reproductive senescence.³¹ Measured by downstream reporters within GSC, TGF- β signaling is reduced in aged females. This decline appears to be functional because the experimental expression of a gene encoding the TGF- β ligand in niche cells starting at young ages increases egg production at late ages. Thus, reproductive senescence to some extent must be caused by declines in the performance of tissues outside of the GSC, and in particular, by changes in the somatic niche.

Like TGF- β , insulin-like peptide is gonadotropic in the fly.^{40, 41, 42} Insulin-like peptide stimulates GSC proliferation in response to food in young adults. The primary source of insulin-like peptides in the adult is brain neurosecretory cells.⁴³ Ablation of insulin-like peptides in the brain of young females retards GSC proliferation.⁴² Likewise, inhibiting the insulin receptor of the gonad retards oogenesis. Because old adults eat less as measured by total uptake of carbon and nitrogen to somatic and reproduction,⁴⁴ oogenesis could be affected by protein intake in two ways. It may reduce the secretion of insulin-like peptides, and it will reduce the supply of nutrients needed to provision developing eggs. The pattern of insulin-like peptide expression with age has yet to be fully documented although insulin

signaling appears to be essential for germline maintenance in females aged for two weeks.^{24, 26, 31, 32} Little is also known about the impact of feeding with age on reproduction except that starvation can induce apoptosis and egg resorption in young flies while oogenesis can be restored by refeeding at advanced ages at the time when previously fed females are already post-reproductive.^{28, 40} Whether these diet-mediated events are caused by the direct limitation of nutrients or through hormonal signals is unknown.

A recent approach to understanding reproductive aging with *Drosophila* used a mutant screen to find genes that increase late age fecundity.²⁷ Transposon insertions that conditionally over-express a unique gene were tested to see if they prevented the age-dependent decline in egg production. Overexpression of two genes increased late fecundity. These genes, named *magu* and *hebe*, also increased adult life span by ~ 5 to 30%. Only *magu* has a recognizable mammalian homolog, which is SMOC2, an extracellular matrix factor known to stimulate cell proliferation and angiogenesis.⁴⁵ The function of *magu* in *Drosophila* is unknown although it often occurs in tissues with stem cells (including the ovary) suggesting as a hypothesis that it may play a role in the maintenance of GSC proliferation.²⁷

Finally, as with *C. elegans*, *Drosophila* is a key tool to understand the relationship between somatic aging, lifespan and reproduction. Past studies using experimental evolution were readily able to increase late age reproduction by direct selection, often at the same time increasing life span and decreasing early reproduction.^{8, 46, 47} While longevity and late reproduction in this context may be evolutionarily constrained by trade-offs with early-age fitness traits, many results also suggest that at least in some environments there is no necessary connection between survival and the schedule of reproduction. Genetic manipulation of several genes such as *indy*, *ecdysone receptor* or *foxo* (the *Drosophila* homolog of *C. elegans daf-16*) can all increase lifespan without reducing either late or early reproduction.^{48–50} Whether these manipulated genes also delay the rate of decline of reproduction with age has not been carefully examined. Conversely, sterility per se does not extend fly lifespan. Mutations of the gene *egalitarian* blocks normal egg chamber development but mutant females are not longer lived than controls⁵¹ and as observed in flies made sterile during development by mutations in the genes *germ cell-less* and *tudor*.⁵² But again as in *C. elegans*, reducing the number of GSC in *Drosophila* is sufficient to extend lifespan.⁵¹ This condition is accompanied by an interesting endocrine response whereby mRNA for insulin-like peptides become highly induced in the fly brain yet insulin signaling in peripheral somatic tissue appears to be very low.

One possible solution to this apparent paradox of hyper-insulin coupled with low insulin response may involve the insulin-like binding protein ImpL2, which is the homolog of mammalian IGFBP7.^{53, 54} ImpL2 binds to and antagonizes insulin-like peptides,^{53, 54} and expression of *ImpL2* is greatly increased in the GSC-knock out adults.⁵¹ A potential explanation for the behavior of this system reasons that insulin-like peptides are gonadotropic and are negatively regulated by some ovary-derived signal that is induced by the germline, much as mammalian FSH is modulated by inhibin derived from follicles. Knockout of the fly germline would release this negative feedback, leading to abundant production of insulin-like peptides. However, while these peptides would be appropriate to stimulate the germline they would also be expected to accelerate somatic aging because reduction of insulin signaling has been shown to increase fly lifespan.⁵⁵ Production of ImpL2 might block the somatic effects of the insulin-like peptide yet still permit activity at the ovary if the hormone-binding protein complex is processed in a tissue specific manner. Analysis of this proposed pituitary-gonad-like axis in *Drosophila* has the potential to model key features of reproductive control that deteriorate with age in mammals.

Conclusions

Despite the differences in the way female reproduction is organized in mammals relative to *Drosophila* and *C. elegans*, we are finding potential parallels in their molecular systems of proliferation and maintenance. Current will aims to better describe the brain/ovary axis of these invertebrates. It will identify signals from the brain that control germline proliferation and, conversely, signals from the gonad that provide feedback to the gonadotropic signals of the brain. It will identify signals from the gonad that directly or indirectly modulate lifespan. And it will likewise further resolve how reduced germline function and egg chamber survival are caused by changes within the germline itself with age, in the somatic environment of the gonad, and most likely in the interaction of these cell populations. These cellular events we suggest, are likely to have conserved properties in the process of mammalian ovarian aging.

References

1. te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum. Reprod. Update.* 2002; 8:141–154. [PubMed: 12099629]
2. Albert Hubbard EJ, Greenstein D. The *Caenorhabditis elegans* gonad: A test tube for cell and developmental biology. *Developmental Dynamics.* 2000; 218:2–22. [PubMed: 10822256]
3. Kadandale P, Singson A. Oocyte production and sperm utilization patterns in semi-fertile strains of *Caenorhabditis elegans*. *BMC Dev. Biol.* 2004; 4:3. [PubMed: 15086962]
4. Hughes SE, et al. Genetic and pharmacological factors that influence reproductive aging in nematodes. *PLoS Genet.* 2007; 3:e25. [PubMed: 17305431]
5. Kenyon C, et al. A *C. elegans* mutant that lives twice as long as wild type. *Nature.* 1993; 366:461–464. [PubMed: 8247153]
6. Larsen PL, Albert PS, Riddle DL. Genes that regulate both development and longevity in *Caenorhabditis elegans*. *Genetics.* 1995; 139:1567–1583. [PubMed: 7789761]
7. Garigan D, et al. Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics.* 2002; 161:1101–1112. [PubMed: 12136014]
8. Bell, G.; Koufopanou, V. The cost of reproduction. In: Dawkins, R.; Ridley, M., editors. *Oxford Surveys in Evolutionary Biology.* Vol. Vol. 3. Oxford: Oxford University Press; 1986. p. 83-131.
9. Motola DL, et al. Identification of ligands for DAF-12 that govern dauer formation and reproduction in *C. elegans*. *Cell.* 2006; 124:1209–1223. [PubMed: 16529801]
10. Magner DB, Antebi A. *Caenorhabditis elegans* nuclear receptors: insights into life traits. *Trends Endocrinol. Metab.* 2008; 19:153–160. [PubMed: 18406164]
11. Shaw WM, et al. The *C. elegans* TGF- β Dauer pathway regulates longevity via insulin signaling. *Curr. Biol.* 2007; 17:1635–1645. [PubMed: 17900898]
12. Savage-Dunn, C. TGF- β signaling. In: WormBook. , editor. WormBook. The *C. elegans* Research Community; 2005. <http://www.wormbook.org>.
13. Ren F, et al. Control of *C. elegans* larval development by neuronal expression of a TGF- β homolog. *Science.* 1996; 272:1389–1391. [PubMed: 8910282]
14. Estevez M, et al. The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. *Nature.* 1993; 365:644–649. [PubMed: 8413626]
15. Matzuk MM, Kumar TR, Bradley A. Different phenotypes for mice deficient in either activins or activin receptor type II. *Nature.* 1995; 374:356–360. [PubMed: 7885474]
16. Lewis KA, et al. Betaglycan binds inhibin and can mediate functional antagonism of activin signaling. *Nature.* 2000; 404:411–414. [PubMed: 10746731]
17. Klein NA, et al. Age-related analysis of inhibin A, inhibin B, and activin a relative to the intercycle monotropic follicle-stimulating hormone rise in normal ovulatory women. *J. Clin. Endocrinol. Metab.* 2004; 89:2977–2981. [PubMed: 15181087]
18. Welt CK, et al. Female reproductive aging is marked by decreased secretion of dimeric inhibin. *J. Clin. Endocrinol. Metab.* 1999; 84:105–111. [PubMed: 9920069]

19. Pierce SB, et al. Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev.* 2001; 15:672–686. [PubMed: 11274053]
20. Boyle M, et al. Decline in self-renewal factors contributes to aging of the stem cell niche in the *Drosophila* testis. *Cell Stem Cell.* 2007; 1:470–478. [PubMed: 18371382]
21. Wallenfang MR, Nayak R, DiNardo S. Dynamics of the male germline stem cell population during aging of *Drosophila melanogaster*. *Aging Cell.* 2006; 5:297–304. [PubMed: 16800845]
22. Cheng J, et al. Centrosome misorientation reduces stem cell division during ageing. *Nature.* 2008; 456:599–604. [PubMed: 18923395]
23. Spradling, AC. Developmental genetics of oogenesis. In: Bate, M.; Martinex-Arias, A., editors. *The Development of Drosophila melanogaster*. Vol. 1.. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1993. p. 1-70.
24. Xie T, Spradling AC. A niche maintaining germ line stem cells in the *Drosophila* ovary. *Science.* 2000; 290:328–330. [PubMed: 11030649]
25. David J, Cohet Y, Fouillet P. The variability between individuals as a measure of senescence: A study of the number of eggs laid and the percentage of hatched eggs in the case of *Drosophila melanogaster*. *Exp. Gerontol.* 1975; 10:17–25. [PubMed: 805709]
26. Zhao R, et al. Age-related changes of germline stem cell activity, niche signaling activity and egg production in *Drosophila*. *Aging Cell.* 2008; 7:344–354. [PubMed: 18267001]
27. Li Y, Tower J. Adult-specific over-expression of the *Drosophila* genes *magu* and *hebe* increases life span and modulates late-age female fecundity. *Mol. Genet. Genomics.* 2009; 281:147–162. [PubMed: 19011900]
28. Good T, Tatar M. Age-specific mortality and reproduction respond to adult dietary restriction in *Drosophila melanogaster*. *J. Insect Phys.* 2001; 47:1467–1473.
29. Kann LM, Rosenblum EB, Rand DM. Aging, oocyte turnover, and the evolution of germline heteroplasmy for mitochondrial DNA length variants in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. USA.* 1998; 95:2372–2377. [PubMed: 9482892]
30. Waskar M, Li Y, Tower J. Stem cell aging in the *Drosophila* ovary. *AGE.* 2005; 27:201–212.
31. Pan L, et al. Stem cell aging is controlled both intrinsically and extrinsically in the *Drosophila* ovary. *Cell Stem Cell.* 2007; 1:458–469. [PubMed: 18371381]
32. Hsu H-J, Drummond-Barbosa D. Insulin levels control female germline stem cell maintenance via the niche in *Drosophila*. *Proc. Nat. Acad. Sci. USA.* 2009; 106:1117–1121. [PubMed: 19136634]
33. Tatone C, et al. Cellular and molecular aspects of ovarian follicle ageing. *Hum. Reprod. Update.* 2008; 14:131–142. [PubMed: 18239135]
34. Wang Z, Lindquist S. Developmentally regulated nuclear transport of transcription factors in *Drosophila* embryos enable the heat shock response. *Development.* 1998; 125:4841–4850. [PubMed: 9806932]
35. Silberman R, Tatar M. Reproductive costs of heat shock protein in transgenic *Drosophila melanogaster*. *Evolution.* 2000; 54:2038–2045. [PubMed: 11209780]
36. Ahn S-G, Thiele DJ. Redox regulation of mammalian heat shock factor 1 is essential for *Hsp* gene activation and protection from stress. *Genes Dev.* 2003; 17:516–528. [PubMed: 12600944]
37. Xie T, Spradling AC. *decapentaplegic* is essential for the maintenance and division of germline stem cells in the *Drosophila* ovary. *Cell.* 1998; 94:251–260. [PubMed: 9695953]
38. Song X, et al. Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, *bag of marbles*, in germline stem cells in the *Drosophila* ovary. *Development.* 2004; 131:1353–1364. [PubMed: 14973291]
39. Chen D, McKearin D. Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. *Curr. Biol.* 2003; 13:1786–1791. [PubMed: 14561403]
40. Drummond-Barbosa D, Spradling AC. Stem cell and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* 2001; 231:265–278. [PubMed: 11180967]
41. Drummond-Barbosa D, Spradling AC. α -Endosulfine, a potential regulator of insulin secretion, is required for adult tissue growth control in *Drosophila*. *Dev. Biol.* 2004; 266:310–321. [PubMed: 14738879]

42. LaFever L, Drummond-Barbosa D. Direct control of germline stem cell division and cyst growth by neural insulin in *Drosophila*. *Science*. 2005; 309:1071–1073. [PubMed: 16099985]
43. Rulifson EJ, Kim SK, Nusse R. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science*. 2002; 296:1118–1120. [PubMed: 12004130]
44. O'Brien DM, et al. Use of stable isotopes to examine how dietary restriction extends *Drosophila* lifespan. *Curr. Biol*. 2008; 18:R155–R156. [PubMed: 18302914]
45. Vannahme C, et al. Characterization of SMOC-2, a modular extracellular calcium-binding protein. *Biochem. J*. 2003; 373:805–814. [PubMed: 12741954]
46. Rose MR. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution*. 1984; 38:1004–1010.
47. Partridge L, Prowse N, Pignatelli P. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Proc. Biol. Sci.* 1999; 266:255–261. [PubMed: 10081162]
48. Rogina B, et al. Extended lifespan conferred by cotransporter gene mutations in *Drosophila*. *Science*. 2000; 290:2137–2140. [PubMed: 11118146]
49. Simon AF, et al. Steroid control of longevity in *Drosophila melanogaster*. *Science*. 2003; 299:1407–1410. [PubMed: 12610309]
50. Hwangbo DS, et al. *Drosophila* dFOXO controls lifespan and regulates insulin signaling in brain and fat body. *Nature*. 2004; 429:562–566. [PubMed: 15175753]
51. Flatt T, et al. *Drosophila* germ-line modulation of insulin signaling and lifespan. *Proc. Nat. Acad. Sci. USA*. 2008; 105:6368–6373. [PubMed: 18434551]
52. Barnes AI, et al. No extension of lifespan by ablation of germ line in *Drosophila*. *Proc. Biol. Sci.* 2006; 273:939–947. [PubMed: 16627279]
53. Honegger B, et al. Imp-L2, a putative homolog of vertebrate IGF-binding protein 7, counteracts insulin signaling in *Drosophila* and is essential for starvation resistance. *J. Biol.* 2008; 7:10. [PubMed: 18412985]
54. Arquier N, et al. *Drosophila* ALS regulates growth and metabolism through functional interaction with insulin-like peptides. *Cell Metab*. 2008; 7:333–338. [PubMed: 18396139]
55. Broughton S, Partridge L. Insulin/IGF-like signaling, the central nervous system and aging. *Biochem. J*. 2009; 418:1–12. [PubMed: 19159343]