REVIEW ARTICLE

Unraveling female reproductive senescence to enhance healthy longevity

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In a society where women often want successful careers and equal opportunities to men, the early nature of ovarian aging often forces women to make difficult life choices between career and family development. Fertility in women begins to decline after the age of 37 years and it is rare for pregnancies to occur after 45. This reproductive decline in women is inevitable and culminates in menopause, which is a major driver of age-related diseases. In a world where biomedical advances are leading to modifiable biological outcomes, it is time to focus on mitigating female reproductive senescence to maintain fertility and preserve age-related hormonal functions, with the goal of providing increased life choices and enhancing healthspan. To date, reproductive longevity research remains an understudied field. More needs to be done to unravel the biology of the ovarian follicles, which are the functional units of reproductive lifespan and are comprised of cell types including the oocyte (female gamete) and a group of specialized supporting somatic cells. Biological attempts to maintain the quality and quantity of follicles in animal models through manipulating pathways involved in aging can potentially prolong female reproductive lifespan and healthspan. Here, we summarize the molecular events driving ovarian aging and menopause and the interventional strategies to offset these events. Developing solutions to female reproductive senescence will open doors to discover ways to enhance true healthy longevity for both men and women.

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INTRODUCTION

In women, the inevitability of reproductive decline is accepted as a natural phenomenon, with consequences on the life choices that are made. Fertility begins to significantly decline after approximately the age of 37 years old (although considerable variation exists) and it is rare for pregnancy to occur after 45 years old. Moreover, menopause is a driver of age-related diseases. In a society where women often want to (and deserve to) have successful careers and equal opportunities to men, prevailing biology often forces choices between sub-optimal outcomes. In a world where biomedical advances are increasingly leading to modifiable biological outcomes, it is time to focus on female reproductive decline as a modifiable event, where maintenance of fertility and preservation of age-related hormonal functions are emphasized with the goal of providing increased life choices and healthspan.

Notably, women live longer than men in most parts of the world — an average of 4–7 years longer in developed countries.[1,2] Yet, the female gonad, the ovary, ages exceptionally early and rapidly before any other parts of the female body system.[3–6] Each woman is born with about 1–2 million oocytes in the form of primordial follicles in her ovaries. After birth, this pool of follicles declines gradually and continuously. This depletion process is accelerated and coupled with a decrease in oocyte quality after about 31 years of age,[7] leading to a gradual loss of fecundity. At the same time, the production of the main ovarian gonadal steroid family, estrogens, declines with the depletion of ovarian follicles. Ultimately, when the number of ovarian follicles drops below 1000,[7] the woman reaches natural sterility, also known as menopause, which occurs at an approximate age of 50 years.[3–6,8,9]

With increased longevity, women spend on average, nearly 40% of their lives in menopause, which is a clinical phenomenon whereby the number of ovarian follicles is so low that there are insufficient levels of estrogens to bring about further ovarian activity and stimulation of the womb lining to result in cyclical menstrual bleeding. This results in the cessation of periods. If a woman aged over 45 years has no spontaneous periods consecutively (and not pregnant) for more than 12 months, she is deemed to have reached clinical menopause. Unfortunately, 1% of all women suffer from a condition known as premature ovarian insufficiency (POI),[10] a state whereby the end of reproductive lifespan occurs before they are 40 years old due to a premature and irreversible loss of ovarian follicles. Importantly, several age-related chronic diseases are disproportionately affected by the onset of menopause.[11,12] For instance, there are heightened risks of neurocognitive decline,[13] cardiovascular diseases (CVDs),[14]

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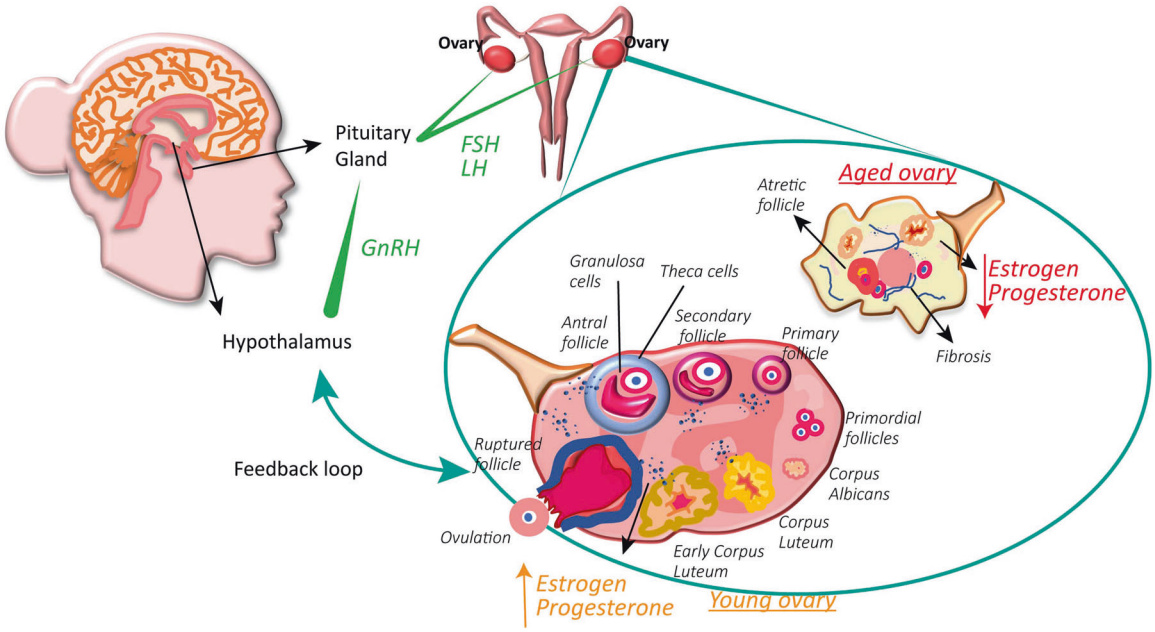

Fig. 1 Ovarian follicles: the functional units of ovary for reproductive lifespan. Along the hypothalamic-pituitary-ovarian axis, the hypothalamus secretes gonadotropin-releasing hormone (GnRH) which travels down to stimulate the pituitary gland which in turn secretes follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH reach the ovaries in the bloodstream to signal the development of ovarian follicles to produce estrogen and progesterone during the follicular and luteal phase of the menstrual cycle. Estrogen rises steadily via a positive feedback loop to result in LH surge from the anterior pituitary gland, leading to ovulation. In a "young ovary", in each cycle, several resting primordial follicles with immature ova are activated. They develop during folliculogenesis and normally only one ovarian follicle will be "selected" as the dominant follicle and eventually releases the mature ovum (ovulation). The ruptured follicle then transforms into the corpus luteum and degenerates to form the corpus albicans if no implantation occurred. Follicular supporting cells such as the granulosa and theca cells, and the corpus luteum provide endocrine support necessary for ovulation, preparation for implantation and pregnancy, with the release of estrogen and progesterone (symbolized as blue dots). As a woman ages, the finite pool of primordial follicles depletes during each ovulatory cycle and along with constant follicular atresia, results in the degeneration and loss of ovarian follicles and their oocytes, becoming the "aged ovary". The "aged ovary" shrinks due to age-related fibrosis and releases little estrogen and progesterone due to the extremely low number of viable ovarian follicles.

metabolic dysfunction,[15] sarcopenia,[16] insulin resistance, osteoporosis[11] and sexual dysfunction. In addition to the permanent loss of fertility, women are at increased susceptibility to cardiometabolic diseases, leading to premature mortality.[14,17]

THE OVARIAN FOLLICLE AS THE BASIC FUNCTIONAL UNIT OF REPRODUCTIVE LIFESPAN AND HEALTHSPAN

Mammalian ovaries, with the ovarian follicles serving as the functional units, work in sync with the mature hypothalamic-pituitary system, known as the hypothalamic-pituitary-ovarian axis, regulate menstrual cycles and govern the reproductive lifespan and healthspan of a woman (Fig. 1). During the menstrual cycle, the ovarian follicles support the maturation of oocytes, which are the female gametes carrying all genetic information and storing nutrients essential for embryo development upon successful fertilization. An enhanced ovarian follicle pool and better follicular quality thus indicate a longer reproductive lifespan.[18]

Ovarian follicles are comprised of somatic cells, known as the granulosa and thecal cells, surround the oocytes and support the growth and development of oocytes. They are the energy hub to produce essential reproductive hormones, progesterone and androgens produced by theca cells and estrogens produced by granulosa cells (Fig. 1).[19] These reproductive hormones, especially estrogens, maintain female reproductive health and provide systemic beneficial effects on other biologic systems. Estrogen is the paramount hormone in regulating the female reproductive system and maintaining women's health, with roles in the cardiovascular system, cognitive function, skin homeostasis and bone metabolism, as reviewed previously.[20–23] Similarly, progesterone, a hormone that is released by the corpus luteum following ovulation and plays a role in maintaining pregnancy, exerts its effects on the reproductive system, but also supports neuroregeneration and neuroprotection.[24]

The number of ovarian follicles is finite at birth in a baby girl, comprising the entire ovarian reserve for her lifetime.[25] As the woman ages, the follicles are constantly recruited for maturation: they either reach ovulation (~400) or (the majority) undergo atresia.[26] Along with this decline in follicle number, follicle quality also decreases with age. This has been proposed to be caused by abnormalities in meiotic spindle assembly and chromosomal distribution in oocytes, as well as mitochondrial dysfunction in both oocytes and the surrounding follicular cells. These biological changes are associated with advancing maternal age and accompanied by declining ovarian steroidogenesis functions due to dysfunctional ovarian folliculogenesis.[27–33] Notably, even in young women with diminished ovarian reserve, the chances of achieving high-quality embryos and successful pregnancy in in vitro fertilization (IVF) have been shown to be much greater compared to older women, despite obtaining similar numbers of eggs.[34] Thus, both quality and quantity of ovarian follicles are important, and their irrevocable decline will result in the end of a woman's reproductive lifespan and, ultimately, a decline in overall health. Therefore, if a woman is born with a very low number of ovarian follicles, or experiences faster depletion of healthy follicles, she will reach menopause earlier. This contrasts with men, who continue to produce spermatozoa (for fertility) and androgens in their testes throughout their lives, albeit with lower quantity and quality as they age.

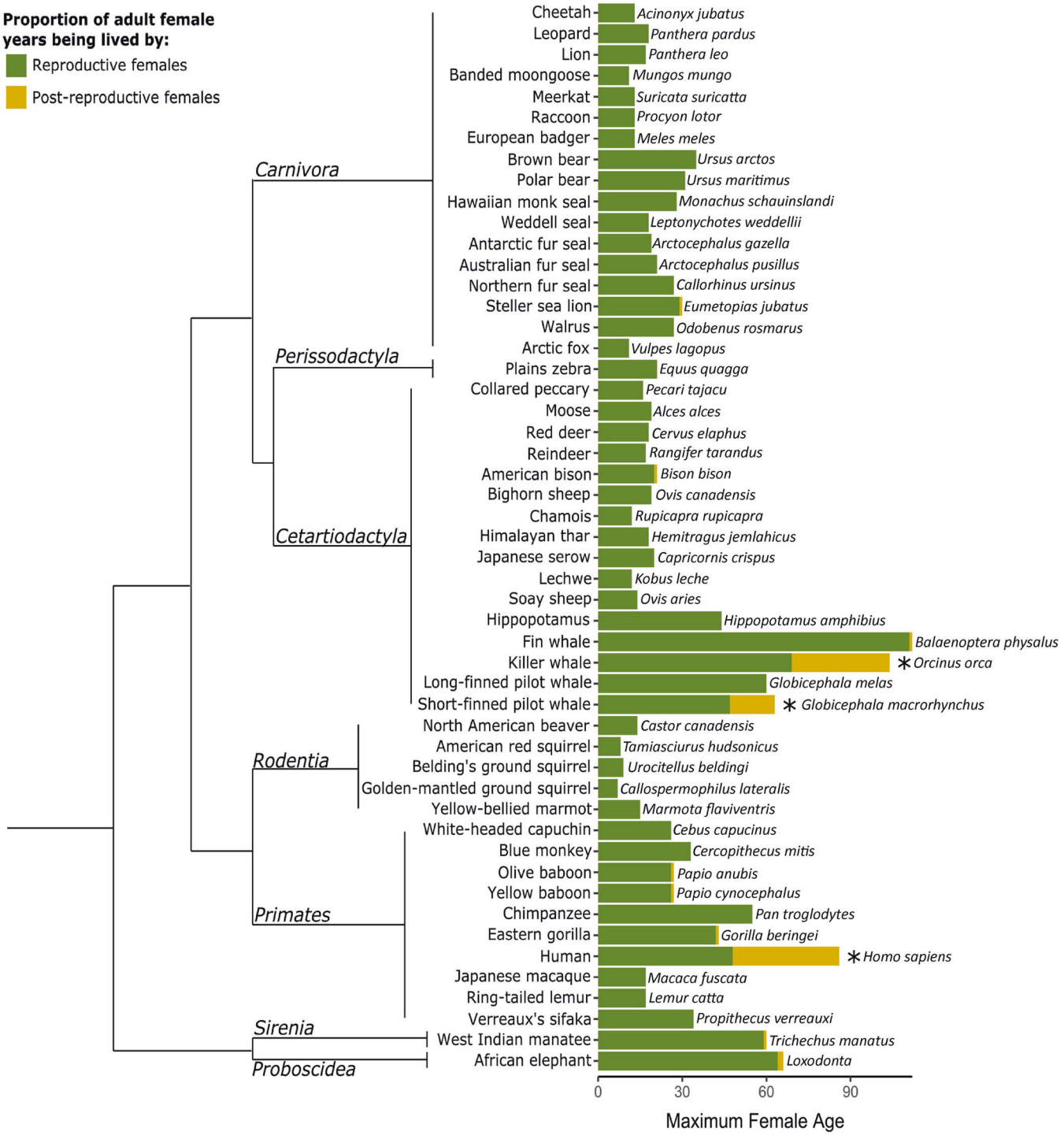

Fig. 2 Post-reproductive lifespan across species.[36] The human female is one of a few selected species of mammals with a significant post-reproductive lifespan. The significance of menopause is extensively being debated on whether it is a vestigial evolutionary inheritance or has crucial implication in conferring evolutionary survival of the fittest to our early days' ancestors as been reproduced from Ellis et al.[36] (Open access license: https://creativecommons.org/licenses/by/4.0/. Image legend has been shifted to the top left of the image from the original image).

MENOPAUSE: AN EVOLUTIONARY VESTIGIAL INHERITANCE?

Menopause appears to decouple reproductive from somatic lifespan in women, leading to reproductive senescence primarily due to a sudden deprivation of serum estrogens.[35] Furthermore, menopause is extremely rare and only known to be present in 2–3 species of mammals; humans being (*Homo sapiens*) is the only terrestrial mammal with menopause (Fig. 2).[36] Interestingly, these species are associated with a longer lifespan as compared to most without a long post-reproductive lifespan.

Beyond the permanent loss of fertility, the hypoestrogenic environment due to menopause is a profound accelerator of aging in women. Importantly, the age of menopause is potentially hereditary, with genome-wide association studies demonstrating the presence of genetic variants linked to menopause that are

involved in DNA repair and maintenance. One genetic variant is also linked to systemic aging.[10] Interestingly, the human ovary, is thought to be the first organ to decline in function with age. The evolutionary history of menopause is thought to be attributed to the "Grandmother Hypothesis",[37,38] whereby energy expended for reproduction is ceased and redirected to tending to their young and future generations to sustain survival of the larger group, as studied in the menopausal female killer whale (*Orcinus orca*).[39] As modern humans have gained longevity due to the shift from prehistoric ancestor roles of hunters-gatherers to current times of technological advancement and enhanced lifespan, menopause may be seen as a vestigial inheritance from our ancestors. Menopause itself, was only coined in the 1820's, by French physician Charles-Pierre-Louis de Gardanne.[40] At that time, the average female lifespan was around 40–50 years of age, which is congruent to what is currently known as age of menopause in women which is ~49–50 years of age.[41]

Until relatively recent times, unhygienic sanitation and lifestyle coupled with the lack of access of quality healthcare, including treatment of infectious diseases, and a range of other factors contributed to early mortality in a non-gender selective manner.[42–45] The relative differences between reproductive lifespan and overall lifespan were likely to be small on an evolutionary timeframe during that period; however, with societal and medical improvements these may have result in large differences between reproductive lifespan and healthspan in women from an evolutionary perspective.

There are selected mammals that have extended reproductive lifespan[46] such as the fin whale (*Balaenoptera physalus*) and elephant (*Loxodonta*) (Fig. 2), with longevity closely linked to the homogametic sex, as compared to the heterogametic sex. Interestingly, however, as compared to humans' closest relatives such as chimpanzees (*Pan troglodytes*),[47] it seems that the duration of reproductive lifespan is somewhat conserved. Yet the lifespan of chimpanzee regardless in the wild or captivity is far shorter.[48] In contrast, prolonged human (*Homo sapiens*) reproductive age is linked to longevity.[49] The conservation of energy due to lower resting metabolic rate during menopause,[50] may have contributed to the longevity in humans, as compared to our closer vertebrate species, while this may not necessarily have translated to healthspan in women with increasing health problems associated with earlier menopause and after the onset of menopause.[41]

Pregnancies in women of advanced maternal age are linked to increased risks of obstetrical complications in the mother, such as gestational diabetes, pre-eclampsia, hypertension, and increased risks of fetal problems such as aneuploidy, e.g., Down's Syndrome and other congenital malformations.[51–55] This is associated with the decline in ovarian follicle quality, which results in oocytes of poorer quality during maternal aging. Therefore, reproductive longevity research would have to address both the extension of reproductive lifespan and the improvement of reproductive healthspan, such that women of advanced maternal age can achieve healthy pregnancies and healthy babies.

REPRODUCTIVE SENESCENCE, HYPOESTROGENISM AND INFLAMMATION AS THE DRIVER OF AGING

Indeed, humans are just a handful of species with an evolutionary divergence of somatic senescence from reproductive senescence, with reaching menopause as the significant driver of aging in women. As discussed above, the deprivation of estrogens from the ovarian follicles dictates this reproductive inevitability. Estrogen confers cardio-protective effects, through improving circulation and vascular health, maintaining oxidative balance, while reducing fibrosis and arterial stiffness in the female vasculature (Fig. 3).[56–61] In preclinical models, estrogen acts as an anti-inflammatory agent, retarding CVD onset and progression, and this appears to be both sexually dimorphic and age sensitive, which explains the lower risk of CVD in premenopausal women compared to men and postmenopausal women.[62] Epidemiological evidence correlating chronic diseases to the onset of menopause suggests that women in the reproductive age group are less prone to these comorbidities when compared to

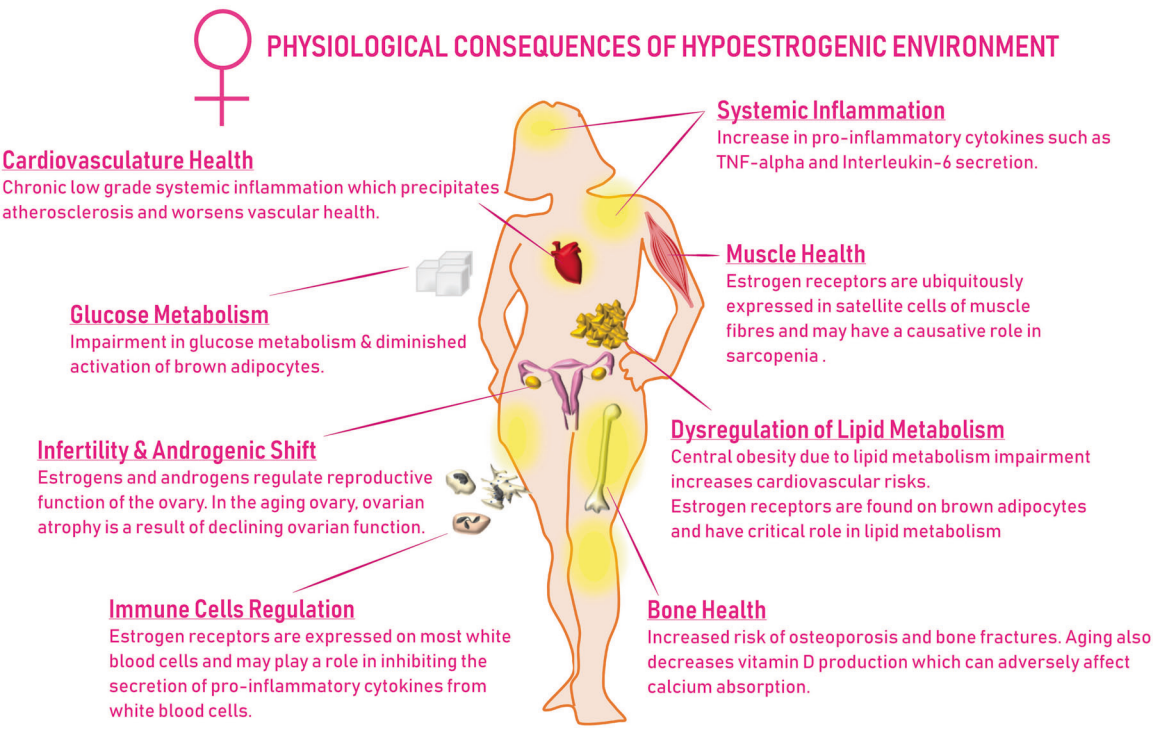

Fig. 3 The post-reproductive lifespan is a result of a hypoestrogenic environment that has pleiotropic health effects. However, the biological significance of the post-reproductive lifespan in women remains unknown.

men.[63–66] Consequently, with the postmenopausal decline in estrogen levels, women become predisposed to chronic inflammatory conditions such as atherosclerosis and type 2 diabetes.[64–69] Estrogen directly binds to the estrogen receptors (ERs), ERα or ERβ,[70] leading to their translocation into the nucleus to target genes driven by promoters containing estrogen responsive elements (EREs) and disrupting the inflammatory cascade by preventing NF-κB transcriptional activation.[71] Disruption of NF-κB has a profound effect on the synthesis and secretion of pro-inflammatory cytokines such as TNFα, IL6, and IL1β. Furthermore, estrogen (exogenous in vivo replacement of 17β-Estradiol, but not progesterone) modulates the cell surface expression of Toll-like receptor 4 (TLR4) in macrophages, which is critical to the pro-inflammatory polarization (M1) phenotype and accompanying cytokine secretion.[72,73]

Reproductive aging and inflammation on musculoskeletal health

Expression of ERs occurs in nearly all musculoskeletal and connective tissues, such as ligament and tendons.[74,75] As the expression of ERs is ubiquitous in satellite cells (adult muscle stem cells) of muscle fibers, estrogen stimulates the activation and proliferation of these quiescent stem cells (satellite cells) during muscle repair or injury.[76] Although both ERα and ERβ are present, ERα contributes to the critical signaling role of muscle stem cell maintenance and muscle regeneration in women.[77] Systematically, chronic inflammation also affects the rejuvenation capability of satellite cells.[78] In contrast, while estrogen is able to improve muscle mass and function, female athletes are known to experience significantly higher incidences of anterior cruciate ligament ruptures (due to increased laxity) during the different phases of the menstrual cycle due to the high estrogen levels, when compared to their male counterparts,[78] whereas, menopause is linked to an elevated risk of musculoskeletal trauma and sarcopenia. This implies that, while estrogen is beneficial directly towards muscle health, it could reduce the stiffness of connective tissues, thereby reducing function and promoting injury.[74]

Bone mass integrity is maintained through the balance between bone resorption by osteoclasts and bone formation by osteoblasts.[79] Osteoporosis is preceded by the micro-architectural loss of bone mass, which gradually reduces the ability of the bone to support body weight. Postmenopausal women are at higher risk of developing osteoporosis, and they form the largest number of osteoporotic cases.[80,81] Serum estrogen levels are directly related to bone mineral density and inversely linked to bone fracture risks.[82] Fundamentally, a hypoestrogenic environment tilts the balance of bone towards resorption, as estrogen exerts profound effects on the development and survivability of both osteoblasts and osteoclasts.[83] Estrogen stimulates the maturation of osteoblasts through upregulation of the TGFβ signaling pathway, which is an apoptotic inducer through Fas ligand in osteoclasts.[79,80,84,85] Furthermore, ERs are highly expressed in osteoblastic lineage cells, with ERα being the more prominent receptor. In ERα-knockout female mice, significant bone loss was observed, a phenotype similar to ovariectomized rodents.[86–89] ERα also confers selective suppression of the receptor activator of NF-κβ (RANKL), a critical cytokine in bone resorption that is secreted by hematopoietic and mesenchymal lineages.[88]

In estrogen deficient states, RANKL upregulation is seen in B lymphocytes.[90] Conditional suppression of RANKL in B lymphocytes but not T lymphocytes, ablated the signs of bone loss in mice.[90] Nonetheless, ERα expression is also found in T lymphocytes and RANKL is secreted from T lymphocytes as well. Although deletion of RANKL from T lymphocytes did little to protect from bone loss, estrogens enhance T cell activation and proliferation.[91] T lymphocytes in return stimulate osteoclast differentiation and bone resorption, while secreting inflammatory cytokines such as TNFα and IL6, which further exacerbate bone loss.[91–93] T lymphocytes therefore could elicit a RANKL-independent, yet synergistic, pathway in osteolysis through the secretion of TNFα.[92] In fact, TNFα is equipotent to RANKL in mediating osteoclastogenesis, further exerting synergistic effects in the presence of RANKL.[92] Collectively, evidence from preclinical studies suggest that estrogen deficiency-induced postmenopausal osteoporosis has a strong immunomodulatory component intertwined with sex steroid actions. Therefore, an immunomodulatory effect from the precursors of T and B cells is likely to be implicated in postmenopausal osteoporosis with RANKL being suggested to be the major contributory factor to hypoestrogenism-induced osteoporosis in bone lining cells.[88]

Calcium makes up the major component in bone that provides strength and structure,[94] and the absorption of calcium from the gut is very much reliant on the availability of vitamin D in the body.[95] Aging is related to an increase in the production of vitamin D[96] but malabsorption of calcium, which has a compounding effect on the onset of osteoporosis.[97] Postmenopausal women suffer a negative calcium balance that slowly stabilizes over the years, but remains tilted towards calcium loss.[98] As natural aging reduces the efficacy of vitamin D production in the liver and skin, it further aggravates the onset of androgenic-induced osteoporosis in women. Therefore, dietary supplementation of vitamin D and calcium, not either or, is only a temporary solution for postmenopausal women at high risk of osteoporosis. Similarly, data from Women's Health Initiative (WHI) studies demonstrate that menopausal hormone replacement therapy (HRT)[99,100] causes a significant reduction in the incidence of bone fractures in postmenopausal women.[99,101] However, the use of menopausal HRT, including transient and lowest dose usage, in mediating benefits on musculoskeletal health needs to be weighed with increased risks of breast cancer and stroke. Several reviews have discussed this issue in more detail.[100,102]

Reproductive aging and inflammation on cardio-metabolic health

Menopause presents a void in sex hormone-mediated homeostasis of glucose and lipid metabolism, which has profound implications in obesity and glucose impairment. Sex hormones are known to regulate the distribution of visceral fat in humans.[103–105] Fat distribution is altered in pre- and postmenopausal women, where subcutaneous fat is progressively being displaced by abdominal fat. Interestingly, adipocytes express both ERα and ERβ, yet only ERα is found to be in brown adipocytes.[106,107] Brown adipose tissue maintains energy expenditure by enhancing lipid and glucose metabolism.[108] It is also part of the endocrine system that secretes adipokines, which regulate inflammatory responses and confer cardio-protection.[109,110] Hence, alteration of ERα in animal models leads to central obesity and diabetes, and in humans this change in fat deposition is also associated with the development of CVD risk factors.[111,112]

During menopause, changes in metabolism include lower energy expenditure, predisposition to central adiposity and an impairment of insulin sensitivity. Clinical evidence is mixed on whether HRT confers CVD protection in postmenopausal women. The Nurse's Health Study (NHS), a prospective longitudinal study spanning 20 years recruited 121,000 female nurses, with 70,533 being postmenopausal, suggested that HRT significantly reduced the risks of CVD, as compared to non-users.[105] In contrast, the Heart and Estrogen/Progesterone Replacement Study (HERS), a randomized controlled trial demonstrated that administering conjugated equine estrogens (CEE) and medroxyprogesterone acetate confers no beneficial effects.[102] Likewise, two subsequent randomized controlled trials of the WHI involving either estrogen + progesterone (WHI E + P) (16,608 postmenopausal women were assessed)[100] or estrogen alone (WHI E)[99] (10,739 postmenopausal women who had undergone hysterectomy were followed) were stopped abruptly following an elevated risk of coronary

heart disease in the former trial and an increase in incidence of stroke in the latter. Successive smaller studies, the Danish Osteoporosis Prevention Study (DOPS)[113] and Early versus Late Intervention Trial with Estradiol (ELITE),[114] were more promising in demonstrating the protective effects of menopausal hormone therapy in reversing the risks of CVD and atherosclerosis. DOPS followed the study population of postmenopausal women for 10 years, and showed that estrogen treatment given to women, significantly suppressed their risks of mortality, CVD, and heart failure. Notably, in DOPS there was no significant elevation of cancer risks.

The optimal period of estrogen rescue is likely to be at the early postmenopausal stage, as demonstrated in ELITE, where there were reduced atherosclerotic events noted at early, but not late stage of postmenopausal intervention. In the Kronos Early Estrogen Prevention Study (KEEPS),[115] only relief of vasomotor symptoms was observed, with a beneficial trend in CVD. In DOPS, ELITE and KEEPS, no obvious adverse effects were observed in the use of estrogens. Findings in ELITE reinforced the "Timing Hypothesis", in which estrogen's Jekyll and Hyde role is dependent on intervention prior to the onset of erosion of coronary plaque, which can be worsened by hormonal therapy.[116] Impairment in glucose metabolism represents a more significant phenotype of menopause. Despite the poor outcome of the HERS and WHI trials in de-escalating the onset of CVD risks, these studies highlighted the significant role that estrogens have in reducing the onset of type-2 diabetes,[99,100,102] a significant risk factor for CVD. Additionally, women who underwent estrogen replacement therapy were observed to have lower hemoglobin A_{1c} (HbA_{1c}), a risk factor for diabetes.[117,118]

Interventions with HRT are directly beneficial to women who suffer from premature POI, while the beneficial effects in women who attain menopause at the expected age of about 50 years likely depend on treatment inception soon after menopause. Potential health benefits by these hormones can be maintained, such as protective effects against CVDs,[119,120] brain aging[121,122] and osteoporosis,[123] thus enhancing the women's healthspan, which is especially true for women who suffer from POI (i.e., menopause before they are 40 years old). It seems that in older women who were more than 5–10 years post menopause and above 60 years of age, HRT can be detrimental and cause increased risks of breast cancer and stroke with long-term use, as demonstrated in the WHI studies, leading to controversies about its use.[124–127] At the same time, several systematic reviews have failed to demonstrate protective effects of HRT against age-related health risks such as CVD events[128] and cognitive decline[129] in postmenopausal women, as HRT does not reverse processes that have already aged. However, it remains essential for women with premature and early ovarian insufficiency who require HRT for maintenance of their healthspan before the age of natural menopause (49–50 years).

The estimated life expectancy has been steadily improved to an average of 72.6 years (and 79.4 years in developed regions) for human beings.[1] However, there has not been an equivalent improvement in healthspan, as the older individuals suffer from frailty and other aging-related medical conditions that affect multiple organs in the body.[130–132] This is further accentuated by ovarian or reproductive senescence. This partial uncoupling of healthspan from lifespan has raised the idea that for people beyond a certain age, i.e., 65 years old, life extension should not be the primary goal.[133,134] Thus, a major need exists to define the critical period for intervention in women to enhance reproductive healthspan and to reduce all-cause morbidity. This likely involves mimicking the beneficial effects of estrogen postmenopausally, while avoiding the associated side effects. This will be one ultimate goal of reproductive longevity research, which will not only attempt to tackle the issue of reproductive lifespan, but also enhance female healthspan and lifespan.

STRATEGIES TO DELAY OR EVEN REVERSE OVARIAN AGING AS MEANS TO MAINTAIN REPRODUCTIVE LIFESPAN AND HEALTHSPAN

Assisted reproductive technology (ART), established in the late 20th century, has become more popular and accessible now[11] to circumvent infertility problems due to the loss of oocyte quantity and/or quality to difficulty in fertilization and embryogenesis. Adjunct strategies, such as oocyte and ovarian tissue cryopreservation, have also matured, leading to rising success rates and utility.[135,136] However, these strategies do have their limitations as they circumvent, instead of directly targeting, the root cause of fertility decline — ovarian aging. Thus, while allowing a woman to try to conceive, these strategies are unable to guarantee reproductive success as oocyte quality constantly decreases with age and advanced maternal age has become the most common factor for IVF failure.[137,138] Furthermore, ART does not restore or maintain the levels and protective effects of ovarian gonadal steroids, such as estrogens.

Given that ovarian follicles are the functional units of ovaries, studies had attempted to enhance ovarian health and prevent reproductive aging through employment of various interventions studied in the context of aging and reported to enhance lifespan in animal models.[139] These studies report molecules/compounds that target specific signaling pathways, spanning from antioxidants, precursors of oxidative stress pathways, mitochondrial chain precursors, isoflavones, polyphenol derivatives, and plant-based compounds, to commonly used medications like metformin and, recently, the mammalian target of rapamycin (mTOR) inhibitors (Table 1). Interestingly, most of this exploratory work was performed in rodent models, and results may differ between these models and humans. Rodent models provide key insights into possible human interventions, but follow-up human studies are needed. This is in part because female rodents and women share progressively increasing irregularity in ovulatory cycles (known as estrous cycle in rodents) and increasing fetal aneuploidy, as decline of oocyte quality and quantity becomes imminent with age, although female mice do not experience menopause like women.[140] Primate models can be employed to study reproductive senescence, although they are expensive and time-consuming.

Utilizing agents studied in the context of general aging for correction of reproductive aging

Referring to Table 1, antioxidants represent the biggest category of compounds studied for reproductive aging, as the free radical theory has been the classical aging theory that attributes aging phenomena to accumulated cellular oxidative stress.[141] According to this theory, the accumulation of reactive oxygen species (ROS) leads to ovarian oxidative stress and changes in ovarian microenvironment, resulting in cellular senescence and a decrease in oocyte quality and quantity.[142–145] Indeed, oxidative stress has been shown to be associated with aging-related oocyte deterioration.[146–148] In addition, previous studies found an increase in ROS and a decrease in levels of antioxidants in oocytes of older women receiving IVF, which are also associated with ART failure.[149–151] Antioxidants such as N-acetyl-L-cysteine (NAC),[152–155] flavonoids,[156] vitamins C and E[157] and coenzyme Q10 (CoQ10) have been tested in rodent models, although many of these agents had other roles in addition to being ROS scavengers (Table 1). Pro-longevity effects of antioxidants in the female reproductive system include maintenance of ovarian reserve, varying improvements of primordial and healthy ovarian follicle counts, a decreased proportion of atretic ovarian follicles and improvement in litter size and estrous cycle regularity. However, the use of antioxidants has also been associated with side effects, including those related to female reproduction, such as long-term disruption of ovarian and uterine functions with pharmacological doses of vitamins C and E.[158] In fact, antioxidants such as vitamins A and E

Table 1. Pharmacological interventions for female reproductive longevity under research in mammalian models and clinical studies.

Pharmacological intervention	Strain and ages/ timings of outcome measurements[a] (all animals used were female)	Dosing regimen	Outcomes at the oocyte level[b]	Outcomes at the ovarian follicle level[b]	Outcomes related to the estrous cycles[b]	Litter size and other mating outcomes[b]	Other outcomes[b]	Clinical evidence and other remarks
NAC[155] NAC is a prodrug of L-cysteine, which is a precursor of the biologic antioxidant glutathione. NAC is thus used as an antioxidant by replenishing the glutathione store. NAC is approved for treating paracetamol overdose and has been widely researched for multiple conditions related to oxidative stress.	– Kunming mice, – At 7–8 months and 12 months of age	Drinking water supplemented with 0.1 mM and 1 mM NAC from 4 weeks of age for 6–12 months	– Decrease in the number of poor-quality oocytes at 12 months of age – No change in the number of ovulated oocytes	NA	NA	– Increase in litter size at 7–8 months of age	– Increase in telomere length and telomerase activity in ovaries	NA
Vitamin C[232] Vitamin C, also known as L-ascorbic acid, is a water-soluble vitamin. Acting as an enzyme substrate and/or cofactor, it is required for the biosynthesis of collagen, L-carnitine, and certain neurotransmitters and involved in protein metabolism. It is also a physiological antioxidant by directly scavenging free radicals and indirectly restoring other antioxidants including α-tocopherol (vitamin E).	– NMRI mice – After 8, 12 and 33 weeks of treatment	Oral gavage of 150 mg/kg vitamin C daily in young adults (25–30 g, age not specified) for 33 weeks	– No change in the total volume of oocytes in antral follicles	– Increase in the number of granulosa cells – Increase in the total number of primordial, primary, and antral follicles at 12 and 33 weeks of treatment	NA	NA	– Increase in ovarian volume	NA
Vitamins C and E[157] The vitamin E group, comprising of tocochromanols (tocopherols and tocotrienols), is the major hydrophobic vitamin exclusively obtained from diet. It reacts with unstable lipid radicals produced in lipid peroxidation to protect cell membranes from oxidative damage.	– F1 hybrid mice (C57Bl/6JIco female × CBA/JIco male) – At the ages of 40–42, 50–52, and 57–62 weeks	Diet supplemented with 10 g/kg vitamin C and 0.6 g/kg vitamin E from first day of weaning or from 32 weeks of age	– Increase in oocyte number – Improvement in oocyte quality by a decrease in age-related chromosomal distribution abnormalities in oocytes	NA	– No effect except a decrease in the number of estrus cycles in the mice treated from weaning in comparison to the mice treated from 32 weeks of age	NA	NA	NA
Genistein[156,233] Genistein is a naturally occurring isoflavone derived from soy products. It exhibits multiple biological activities including anti-aging, antioxidant, phytoestrogenic and antineoplastic activities.	– Sprague-Dawley rats – At 15 months of age[156]	Intragastric gavage of 160 mg/kg genistein daily from 11 months of age for 4 months	NA	– Increase in the number of healthy follicles – Decrease in the number of atretic follicles – Increase in the number, but no change in the percentage of primordial	– Maintenance of estrous cycle	NA	NA	In a study[234] feeding adult female cynomolgus monkeys at 9–15 years of age saturated fat and cholesterol-containing diets, the diet with soy protein source containing genistein

Table 1. continued

Pharmacological intervention	Strain and ages/ timings of outcome measurements[a] (all animals used were female)	Dosing regimen	Outcomes at the oocyte level[b]	Outcomes at the ovarian follicle level[b]	Outcomes related to the estrous cycles[b]	Litter size and other mating outcomes[b]	Other outcomes[b]	Clinical evidence and other remarks
				follicles – Decrease in the percentage of primary follicles				has led to higher numbers of primary and secondary follicles compared to the other diet with casein-lactalbumin source.
	– Sprague-Dawley rats – At 4 months and 15 months of age[233]	Intragastric gavage of 160 mg/kg genistein daily from 3 months of age for 4 weeks and 11 months of age for 4 months	NA	– Increase in the number of healthy follicles – Decrease in the number of atretic follicles – Increase in the numbers of primordial and antral follicles at 15 months of age	– Maintenance of estrous cycle	NA	NA	
Resveratrol[235,156,167] Resveratrol is a natural polyphenol derived from red grapes and found in several other plants. It has been widely investigated as a therapeutic treatment for multiple diseases due to its antioxidant, anti-aging, anti-inflammatory, and anti-neoplastic properties. Resveratrol has been shown to scavenge ROS, enhance antioxidant biosynthesis and induce sirtuin-1 signaling.	– Sprague-Dawley rats – At 15 months of age[156]	Intragastric gavage of 25 mg/kg resveratrol daily from 11 months of age for 4 months	NA	– Increase in the number of healthy follicles – Decrease in the number of atretic follicles – Increase in the number, but no change in the percentage of primordial follicles – Decrease in the percentage of primary follicles	– Maintenance of estrous cycle	NA	NA	A cross-sectional retrospective study[167] showed a strong association between resveratrol oral supplementation (200 mg/day) in women during IVF-embryo transfer and a decrease in clinical pregnancy rate.
	– C57 mice – At 14–16 months of age[235]	Drinking water supplemented with 30 mg/L resveratrol from 6 weeks of age for 6–12 months	– Increase in oocyte number – Improvement in oocyte quality by better spindle morphology and chromosome alignment	– Increases in the numbers of primordial and primary follicles, as well as secondary and antral follicles – No change in atretic follicle number	NA	– Increase in litter size	– Prevention of telomere shortening and improvement in telomerase activity in ovaries	
CoQ10[237] CoQ10, also known as ubidecarenone, is a naturally occurring component in cell membranes. It is an essential electron carrier in mitochondrial respiratory chain. CoQ10 also functions as an antioxidant via inhibition of lipid peroxidation as well as protein and DNA oxidation.	– ICR mice – At 12 months of age	Subcutaneous injection of 22 mg/kg CoQ10, three times a week from 9 months of age for 12–13 weeks	– Improvement in oocyte quality by reduction in oocyte spindle defects – Improvement in oocyte mitochondrial function	– Increase in the numbers of primordial, primary, and secondary follicles	NA	– Increase in litter size	– Increase in ovulation rate	In the same study,[236] resveratrol (10 mg/kg) or lipoic acid (33 mg/kg) using the same dosing regimen and mouse strain did not lead to an increase in the ovulation rate.

Table 1. continued

Pharmacological intervention	Strain and ages/ timings of outcome measurements[a] (all animals used were female)	Dosing regimen	Outcomes at the oocyte level[b]	Outcomes at the ovarian follicle level[b]	Outcomes related to the estrous cycles[b]	Litter size and other mating outcomes[b]	Other outcomes[b]	Clinical evidence and other remarks
Apocynin[237,238] Apocynin, also known as acetovanillone, is a plant-derived polyphenol with multiple biological activities. It can suppress superoxide production by inhibiting NADPH oxidase.	– C57BL/6 J mice – At 15–19 and 35–49 weeks of age[237]	Drinking water supplemented with 5 mM apocynin from 8–12 weeks or 38–42 weeks of age for 7 weeks	NA	– No change in the number or percentage of follicles at different stages	NA	NA	– Improvement in redox homeostasis and reversal of age-related protein carbonylation increase in ovaries and uteri – Normalization of age-related inflammation factors and collagen expression in ovaries	NA
	– C57BL/6 J mice – At 8–12 weeks and 38–42 weeks of age[238]	Drinking water supplemented with 5 mM apocynin from 6 weeks of age until 8–12 weeks or 38–42 weeks of age	NA	NA	NA	– Increase in litter size – Increase in the number of uterine implantation sites	– Improvement in redox homeostasis and reversal of age-related protein carbonylation increase in uterus	
Acetyl carnitine and lipoic acid[239] Both acetyl carnitine and lipoic acid are natural compounds in the body and have demonstrated anti-inflammatory and antioxidative properties. Acetyl carnitine is formed by the addition of an acetyl group to the amino acid carnitine while lipoic acid is derived from octanoic acid. Acetyl carnitine facilitates fatty acid metabolism while lipoic acid has been shown to increase glutathione synthesis and regenerate vitamins C and E.	– C57BL/6 mice – After 3, 6, 9 and 12 weeks of treatment	Drinking water supplemented with 100 mg/L acetyl carnitine and 40 mg/L lipoic acid from 3 weeks of age for 3, 6, 9 or 12 months of treatment	– Increase in oocyte number – Improvement in oocyte quality by reduction in oocyte chromosomal and spindle abnormalities – Improvement in oocyte mitochondrial function	NA	NA	NA	NA	NA
Dimethylfumarate (DMF)[240] DMF is a lipophilic drug approved for treating psoriasis and sclerosis. It has been shown to activate the Nrf2 pathway and possess immunomodulatory and antioxidant properties.	– BALB/c mice – At 48 weeks of age	Oral administration of 50 mg/kg DMF daily from 32 weeks of age for 16 weeks	– Increase in oocyte number	– Increase in the number of primordial follicles – No change in the numbers of primary, secondary, and antral follicles	NA	NA	– Elevation of serum AMH – Increase in antioxidant levels and decrease in oxidative stress in ovaries – Increase in telomere mRNA and protein levels in ovaries	NA
Catalpol[241] Catalpol, a plant-derived iridoid glucoside, has been shown to have antioxidative, anti-	– Sprague-Dawley rats – At 15 months of age	Oral gavage of 1, 3 or 5 mg/kg catalpol daily from 14 months of age for 4 weeks	NA	NA	NA	NA	– Alleviation in ovarian weight loss and structural abnormalities	NA

Table 1. continued

Pharmacological intervention	Strain and ages/ timings of outcome measurements[a] (all animals used were female)	Dosing regimen	Outcomes at the oocyte level[b]	Outcomes at the ovarian follicle level[b]	Outcomes related to the estrous cycles[b]	Litter size and other mating outcomes[b]	Other outcomes[b]	Clinical evidence and other remarks
inflammatory, anti-apoptosis, and neuroprotective properties.							– Rejuvenation of ovarian granulocytes – Increase in serum estradiol and progesterone levels but decrease in serum follicle-stimulating and luteinizing hormone levels	
Metformin[177,179,242] Metformin is a first-line biguanide antidiabetic drug. It has been shown to reduce hepatic glucose production and intestinal glucose absorption and improve insulin sensitivity. Metformin is also used to treat polycystic ovarian syndrome and investigated as a longevity drug. Proposed mechanisms of its life-extending effects include activation of the AMPK pathway, as well as modulation of the gut microbiota and DNA methylation.	– C57BL/6 – At 54 weeks of age[177]	Diet supplemented with 100 mg/kg metformin from 28 weeks of age for half a year	NA	– Increase in the numbers of primordial and primary follicles	– Maintenance of estrous cycle	NA	– Reduction in ovarian oxidative damage and senescence marker P16 – Maintenance of serum estradiol level	A prospective randomized trial[242] found that female IVF repeaters without PCOS and with a mean age of about 39 years who took 500 mg/day metformin for 8–12 weeks before and during ovarian stimulation have higher ongoing pregnancy and implantation rates compared to those untreated.
	– Wistar albino rats – At 12 weeks of age[179]	Oral gavage of 100 and 200 mg/kg/day metformin from 8 weeks of age for four weeks	NA	– No significant change in the number of primordial, primary, or total follicles, except an increase in secondary follicle count with 200 mg/kg/day dose	NA	NA	– Decrease in endometrial thickness	
2-DG[180] 2-DG is a glucose derivative that competitively inhibits glycolysis, thus mimicking glucose restriction. It is also an investigational anticancer and antiviral drug.	– C57BL/6 mice – At 7 weeks of age	Intraperitoneal injection of 100, 300 or 600 mg/kg 2-DG daily from 5 weeks of age for 2 weeks	NA	– Reduction in follicular activation by a decrease in the type 3a primary follicle count	NA	NA	NA	NA
NAD^+ boosters[186,187] NAD^+ is naturally synthesized in the body. It is a central coenzyme in energy metabolism and an essential redox cofactor. The NAD^+ level decreases with age and NAD^+ repletion via the use of NAD^+ precursors has been investigated as a therapeutic approach in multiple age-associated conditions, such as cardiovascular and neurodegenerative diseases	– Mainly C57BL/6 mice – At 12–16 months of age[187]	Drinking water supplemented with 0.5 or 2 g/L of nicotinamide mononucleotide from 12 or 13 months of age for 4 weeks	– Improvement in oocyte quality by better oocyte spindle assembly – Increase in oocyte yield, oocyte diameter and blastocyst formation rates (2 g/L)	NA	NA	– Increase in litter size – Increase in pregnancy rate and live birth rate (0.5 g/L)	NA	NA
	– C57BL/6 mice – At 12 months of age[186]	Drinking water supplemented with 400 mg/kg/day of nicotinamide riboside from	– Improvement in oocyte quality by better oocyte spindle assembly	– Increase in the numbers of primordial, primary, and total follicles	– Maintenance of estrous cycle	– Increase in litter size	– Increase in ovary size and the number of ovulated cumulus–oocyte	

Table 1. continued

Pharmacological intervention	Strain and ages/ timings of outcome measurements[a] (all animals used were female)	Dosing regimen	Outcomes at the oocyte level[b]	Outcomes at the ovarian follicle level[b]	Outcomes related to the estrous cycles[b]	Litter size and other mating outcomes[b]	Other outcomes[b]	Clinical evidence and other remarks
		8 months of age for 4 months	– Improvement in mitochondrial functions in ovaries and oocytes				complexes after superovulation	
α-ketoglutarate (AKG)[243] AKG is a key intermediate in the Krebs cycle, a nitrogen scavenger and a precursor of glutamate and glutamine. AKG can enhance protein synthesis, bone development and immune responses. It has also demonstrated life-extending effect by inhibiting ATP synthase and TOR.	– ICR mouse – At 8 and 14 months of age	Drinking water supplemented with 2, 10, 25, and 50 mM (optimal: 10 mM) AKG from 2 to 14 months of age	– Increase in oocyte number – Improvement in oocyte quality by reduction in abnormalities in mitochondrial distribution and spindle arrangement	– Increase in the number of primordial and primary follicles – No significant change in the numbers of secondary, mature and atretic follicles	– No significant effect in estrous cycle	– Increase in litter size, pregnancy rate and pregnancy outcomes – Decrease in neonatal death rate	– Reduction in telomere shortening	NA
Melatonin[218–229] Melatonin is an amine neurohormone primarily secreted by pineal gland at night. Besides its role of maintaining circadian rhythm, melatonin also acts as a potent antioxidant and a direct free radical scavenger, speculatively through the Nrf2-ARE pathway and sirtuin activation. Moreover, melatonin has been shown to increase lifespan in some animal models.	– Holtzman rats – At 75, 180 and 380 days of age[218]	Drinking water supplemented with 10 μg/mL melatonin from 10 days of age, either only at night or continuously for one year	NA	– No change in the number of primordial follicles	– Decrease in estrous cycles with abnormal length with treatment only at night	NA	NA	Several clinical trials[224–229] have investigated the effects of oral administration of melatonin, usually at the dose of 3 mg daily, in women with infertility or a history of IVF failures. Positive changes of gonadotropins, decrease in degenerate oocytes, improvement in oxidative balance and oocyte quality and/or a slight increase in IVF fertilization rate have been found in some studies,[224–226,229] However, other studies,[226–228] including one using a high dose of 8 mg twice daily, have failed to demonstrate an increase in clinical pregnancy rate, live birth rate or quality or quality of oocytes during IVF.
	– Wistar rats – At a) 5, b) 15 and c) 24 months of age[219]	Subcutaneous injection of 150 μg/ 100 g daily from a) 3, b) 13 and c) 22 months of age for two months	– b) Increase in oocyte volume – a) b) c) No change in oocyte density	NA	– b) Improvement in estrous cycle regularity	NA	– b) Increase in ovarian volume – a) c) No change in ovarian volume – a) b) Maintenance of serum estradiol level	
	– Kunming mice – At 14–16 months of age[221]	Drinking water supplemented with 10 mg/kg melatonin from 2–3 months of age for 6–12 months	– Increase in oocyte number – Improvement in oocyte quality by better spindle morphology and chromosome alignment	– Increase in primordial follicle count – Decrease in atretic follicle count – No change in growing or mature follicle counts	NA	– Slight increase in litter size	– Improvement in ovarian mitochondrial antioxidant activity – Increase in telomere length – Suppression of ovarian apoptosis	
	– ICR mice – At 43 and 53 weeks of age[220]	Drinking water supplemented with 100 μg/mL melatonin from 10 to 43 weeks of age	– Increase in the number of ovulated oocytes	– Increase in numbers of primordial, primary, and antral follicles	NA	NA	– Increase in telomere length – Increase in rates of fertilization and blastocyst formation following IVF	
	– Kunming mice – At 24–48 weeks of age[222]	Drinking water supplemented with 10−3, 10−5, 10−7 mol/L (optimal: 10−5 mol/L)	– Increase in oocyte number – Improvement in oocyte quality by improved	NA	NA	– Increase in litter size	– Improvement in ovarian antioxidant capacity – Increase in the number of	

Table 1. continued

Pharmacological intervention	Strain and ages/ timings of outcome measurements[a] (all animals used were female)	Dosing regimen	Outcomes at the oocyte level[b]	Outcomes at the ovarian follicle level[b]	Outcomes related to the estrous cycles[b]	Litter size and other mating outcomes[b]	Other outcomes[b]	Clinical evidence and other remarks
		melatonin from 8 to 24–48 weeks of age	morphology and blastocyst formation – Reduction of ROS formation in oocytes				formed blastocysts and litter size following IVF	
	– Kunming mice – On a) postnatal day 6 and 9 and b) postnatal day 15, 17, 19 and 21[223]	Injection of 1 and 15 mg/kg melatonin daily a) from postnatal day 3 to 9 and b) from postnatal day 10 to 21	– b) No change in oocyte diameter – b) Decrease in oocyte number after superovulation	– a) Decrease in the number of activated follicles, but no change in atretic follicle number – b) Decrease in the numbers of type 5b follicles and atretic follicles	– b) No change in estrous cycle	– b) Decrease in the number of implanted embryos with bigger embryo size	– a) Improvement in ovarian antioxidant capacity – b) Decrease in *Fshr* and *Lhcgr* gene expressions	
Rapamycin[4,6,207,208,210,244] Rapamycin, also known as sirolimus, is a natural anti-fungal macrolide produced by the bacterium *Streptomyces hygroscropicus*. Currently approved as an immunosuppressant, it has demonstrated therapeutic potential in other aspects, such as antiproliferation and immunomodulation in cancer treatment. Rapamycin has also been shown to extend both lifespan and healthspan in multiple animal models by inhibiting the target of rapamycin (TOR), a highly conserved key protein kinase regulating metabolism and physiology.	– C57BL/6 mice – At 8 weeks of age[244]	Intraperitoneal injection of 2 doses of 5 and 50 mg/kg rapamycin from 8 weeks of age for two consecutive days	– Reduction in ovulated eggs with unaffected quality	– No change in follicle reserve	NA	NA	NA	NA
	– Sprague-Dawley rats – At 20 weeks of age[207]	Intraperitoneal injection of 5 mg/kg rapamycin every other day from 10 weeks of age for 10 weeks	NA	– Suppression of primordial follicle activation by an increase in primordial follicle number – Decrease in numbers of antral and atretic follicles and corpora lutea	– Disturbance of estrous cycle, during or just after treatment	NA	– Reduction in ovarian weight	
	– Adult rats – Age not specified[210]	Intraperitoneal injection of 5 mg/kg rapamycin every other day for 10 weeks	NA	– Suppression of primordial follicle activation by an increase in primordial follicle number – Decrease in numbers of antral and atretic follicles and corpora lutea	– Disturbance of estrous cycle, during or just after treatment	– Failure of being impregnated	NA	
	– C57BL/6 mice – At a) postnatal day 7 or b) postnatal day 21[208]	Intraperitoneal injection of 1 mg/kg rapamycin a) on postnatal day 2 and b) from postnatal day 7 and then every other day	NA	– Suppression of primordial follicle activation by an increase in primordial and transitory	NA	NA	NA	

Table 1. continued

Pharmacological intervention	Strain and ages/ timings of outcome measurements[a] (all animals used were female)	Dosing regimen	Outcomes at the oocyte level[b]	Outcomes at the ovarian follicle level[b]	Outcomes related to the estrous cycles[b]	Litter size and other mating outcomes[b]	Other outcomes[b]	Clinical evidence and other remarks
		until postnatal day 21		follicle numbers and decrease in the numbers of primary and activated follicles				
	– CD1 mice – Just after treatment, 2 months after treatment, and 16 months of age[4]	Intraperitoneal injection of 2 mg/ kg rapamycin daily from 8 weeks or 8 months of age for 2 weeks	– No change in oocyte number – Improvement in oocyte quality by morphology and spindle arrangement	– Suppression of primordial follicle activation by an increase in primordial follicle number and decrease in the numbers of primary, secondary, and antral follicles	– Disturbance of estrous cycle, during or just after treatment – Re-normalization and maintenance of estrous cycle and hormone biogenesis 2 months post treatment	– Improvement in late-life fecundity	NA	
	– C57BL/6 mice – At 130 days of age[6]	Intraperitoneal injection of 4 mg/ kg rapamycin every other day from 37 days of age for 93 days	NA	– Suppression of primordial follicle activation by an increase in primordial follicle number and decrease in the numbers of primary, secondary, and tertiary follicles	NA	NA	NA	

[a]In vivo studies on aged wild-type animals and clinical studies treating infertile women without a particular confounding disease (e.g., PCOS, POI).
[b]Outcomes in comparison with untreated age-matched control in each study unless otherwise stated.

were shown to increase mortality in clinical trials.[159] This may be explained by potential beneficial functions of oxidative stress in some contexts, for example, reduction of insulin resistance triggered by exercise-induced ROS.[160] Additionally, several systematic reviews failed to demonstrate any positive outcome of antioxidant supplementation in the context of general aging[159] and other age-related diseases, such as cataract, dementia and CVD.[161–164] In the context of ovarian aging, the in vivo studies on antioxidants were almost exclusively done in relatively young or middle-aged animals and failed to assess the effects in more aged ovaries (from older animals), potentially limiting the translatability to older or even postmenopausal women. Furthermore, only a few antioxidants have been studied in women with age-related ART failure and the results remain highly inconsistent.[165,166] The oral supplementation of some antioxidants such as resveratrol was even associated with decreased pregnancy rates, the converse of what was expected.[167] Reservatrol also has many aging-associated actions that are independent of its antioxidative effects,[168] further complicating the interpretation of these findings.

Manipulation of glucose metabolism to mimic the effects of caloric restriction (CR) has been a focus in longevity studies. CR, the chronic reduction in total calorie intake without malnutrition, robustly improves both healthspan and lifespan in many organisms.[169] As CR is not easy to be implemented in real life, genes and pathways involved in longevity mechanisms of CR are being identified and there are multiple candidate compounds that can potentially mimic the longevity effects of CR. Metformin, an approved anti-diabetic drug, achieves a reduction in blood glucose by enhancing peripheral insulin sensitivity and suppressing gluconeogenesis in the liver,[170,171] which resemble some metabolic effects of CR.[172] Indeed, metformin has demonstrated promising results in enhancing healthspan and lifespan in animal models[172,173] and clinical trials.[174] In the context of ovarian aging, multiple studies revealed that CR preserved oocyte quality, fertility and/or ovarian reserve in aged female rodents.[6,175,176] Similarly, a recent study reported that six months of metformin treatment increased serum estrogen level and follicle quantity and resulted in more regular estrous cycles in normally aged mice.[177] Such effects are consistent with another study showing an association between fasting-induced lower blood glucose level and reduced primordial follicular activation in mice.[178] However, this observation was not corroborated by another rodent study,[179] which found comparable follicle counts between metformin-fed rats and control rats. As there were differences in duration of treatment and rodent species, further investigations are needed to assess the effects of metformin in mammalian ovaries. Importantly, older animals need to be used to examine the true biological effects, as the previous studies were conducted on young rodents. These limitations apply to another study investigating 2-deoxyglucose (2-DG), where young mice and a short treatment duration were employed.[180] 2-DG is a synthetic glucose analog that competitively inhibits glycolysis, reduces insulin levels and decreases body temperature in rats, and thus has also been considered as a candidate CR mimetic.[181] Interestingly, this study[180] revealed inhibition of primordial follicle activation by 2-DG, indicating its potential in preservation of primordial ovarian follicles — hence protecting the ovarian reserve. However, the chronic treatment of 2-DG has been shown to cause cardiotoxicity and increased mortality in male rodents.[182] Although it remains unknown whether this is also the case in females, such toxicity has decelerated the transition of 2-DG to clinical use.

Mitochondrial dysfunction has been identified as a hallmark for aging and implicated in ovarian aging and infertility, as oocytes are uniquely enriched with mitochondria.[183] Aging-related changes in mitochondria, including accumulation of mitochondrial DNA mutations, altered membrane potential and impaired metabolism, undermine mitochondrial functions and are proposed to link to ovarian aging phenotypes.[184] Besides the aforementioned antioxidant therapies, such as CoQ10, a combination of vitamins C and E, and flavonoids, aimed to increase nicotinamide adenine dinucleotide (NAD^+), an essential cofactor and enzyme substrate in several crucial redox reactions and metabolic pathways which declines with age,[185] has been shown to alleviate ovarian aging by improving mitochondrial function. NAD^+ supplementation was tested in two recent studies in young and middle-aged mice.[186,187] These studies demonstrated that the oocytes were rejuvenated, with enhanced fertility attributable to a reduction in levels of ROS and improvement in ovarian mitochondrial metabolism.[186] However, knowledge about mitochondrial boosters including NAD^+ precursors remain limited as they were only tested in rodents and in ages up to 14 months in mice. Therefore, mitochondrial boosters are likely to gain more attention in the future, especially given the new findings linking mitochondrial dysfunction to female reproductive aging via impaired NADH/NAD^+ redox functions.[188] Notably, the sirtuins, a family of NAD^+-dependent deacylases and key regulators of aging, have both mitochondrial and non-mitochondrial functions such as DNA repair and inflammatory response.[189] Thus, the mechanism of NAD^+ supplementation in ovarian longevity may be pleiotropic.

mTOR is a serine/threonine protein complex that is sensitive to rapamycin and mTOR suppression has been shown to extend lifespan in several species.[190–199] mTOR also coordinates several key cellular signaling and metabolic pathways implicated in follicular development and ovarian aging.[200] Follicular mTOR signaling stimulates primordial follicle activation, which is the start of post-puberty follicular development and directly determines the follicular reserve and reproductive lifespan. It was demonstrated in vivo that ovarian mTOR overactivation triggers premature follicular activation and early follicle depletion,[201–205] together with more atretic follicles and degenerated oocytes.[205] At the same time, AKT-mediated mTOR signaling was found to regulate granulosa cell autophagy in folliculogenesis and its inhibition was found to induce follicular atresia through promoting granulosa cell autophagy.[206] Studies in rodent models[4,207–210] have demonstrated that inhibition of mTOR signaling can improve ovarian reserve, as indicated by the increase in primordial follicle counts and extension of reproductive lifespan. There has also been some evidence indicating a decrease in the absolute atretic follicle count,[210,211] probably due to overall suppression of follicular activation. Unfortunately, rapamycin was also found to cause disruption of estrous cycles and loss of fertility, due to a cessation of follicle activation after prolonged use of more than 4 months. This was expected because mTOR signaling plays a strong role in follicular growth as discussed. Nevertheless, this disruption seems to be reversible, as a recent study[4] devised a 2-week transient rapamycin treatment that successfully restored follicular development and estrus cycles in post-treatment mice, observing an improvement in the treated mice's reproductive capacity and ovarian lifespan, regardless of their ages at treatment up to 16 months of age. This approach may be consistent with the longevity effects of rapamycin, where a transient treatment even in relatively late age is sufficient to extend healthspan and lifespan.[211] mTOR inhibitors could potentially be useful in the treatment of reproductive aging, but further studies are required due to considerations on utilizing mTOR inhibitors as strategic short-term treatment modality to achieve long-term protective effects against ovarian aging in women. Apart from inhibition, as mTOR plays a crucial role in reproduction, its chronic activation in reproductive aging should be extensively examined with small molecules such as MHY1485[212] and 3BDO,[213] which are potent mTOR activators.

Other options such as dehydroepiandrosterone (DHEA), a precursor for estrogen in peripheral tissues, and melatonin, a sleep-promoting hormone with antioxidant properties, have shown some success in extending reproductive lifespan and/or improving ovarian responses in both animal and clinical studies.

The concentration of DHEA decreases progressively with age.[214] As an essential prohormone in ovarian steroidogenesis, DHEA was suggested to promote gonadotropin action and ovarian functions, as indicated by the improvement in anti-Mullerian hormone (AMH) level and IVF outcomes in women with diminished ovarian reserve.[215,216] This is further supported by an ovine study that also showed an increase in follicular AMH expression and granulosa cell proliferation after DHEA treatment.[217] Yet, the same study also found an accelerated primordial follicle activation and an increase in antral follicle count, which raises the concern of pre-mature follicle depletion especially upon long-term administration. In contrast, melatonin, also an endogenous hormone in the body, has more extensive evidence in delaying ovarian aging. In rodents, it was found to improve oocyte quality and quantity and maintain follicular reserve.[217–224] A recent study[223] demonstrated the endogenous role of melatonin in suppressing follicular over-activation and atresia and delaying ovarian aging. It was proposed that melatonin acts through its antioxidant capacity and the MT1/AMPK pathway,[220,221,223] though further mechanistic study is needed. However, these studies varied greatly in age of subjects, end-point measurements and duration of treatment. For instance, supplementation of melatonin was conducted mostly on very young mice daily for half to one year,[218,220–222] which makes the translation of this treatment in clinical settings impractical due to the widespread metabolic and physiological activities that melatonin exerts on the body, raising concerns on any unanticipated adverse effects which can occur due to prolonged administration. Additionally, several clinical trials investigating oral melatonin administration in women with infertility or IVF failures have demonstrated conflicting results, which are summarized in Table 1.[224–229]

A list of compounds tested in this context are listed in Table 1. However, many of the studies are primarily observational and although many compounds are purported to "extend reproductive lifespan", results were often derived from findings in relatively young animals treated for varying durations. Furthermore, the exact mechanisms of actions for these interventions still lack clarity, and the therapeutic targets as well as their sites of action are yet to be elucidated. There remains a lot to be studied in understanding the underlying biological mechanisms governing reproductive aging at the ovarian follicle level and in the surrounding ovarian environment.

FUTURE DIRECTIONS

There is a compelling need to develop new strategies to offer women to:

(1) Choose when she can fulfill her hopes of childbearing, once the mysteries behind ovarian senescence are better understood.
(2) Protect and enhance her reproductive lifespan and healthspan using innovative solutions that are safe, with minimal or no side effects, to overcome the negative consequences of reproductive aging.
(3) Advocate for reproductive longevity and equality in women, and for science to assist in tackling the inevitability of reproductive aging for future generations.

New intervention strategies should target the root cause of the reproductive aging process, ideally at a specific organ-cell-molecular target level, and be relatively short-term and have minimal side effects.

CONCLUSION

Current knowledge on the mechanisms of ovarian or reproductive aging remains limited. Clinical management of fertility issues is always confounded by maternal age with reproductive outcomes limited by the age of women, i.e., older age and reduced ovarian reserves result in higher risks of reproductive failure.[230] Few options are available for the management of women with POI and natural menopause because we still do not fully understand the biology of ovarian senescence in women. The balance between reproductive lifespan (fertility) and healthspan (general good health) will be the long-term goal and imperative as part of the movement for gender equality. A woman should not be limited to her reproductive lifespan and "accept" what biology has imposed on her reproductive choices and life choices, in addition to her decline of healthspan later in life. Women represent half of the world population, and they also carry the future of the world. Reproductive longevity in women also determines healthy longevity in their male relatives,[231] which emphasizes the importance of solving female reproductive senescence and will open doors to discover ways to enhance true healthy longevity for both men and women.

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AUTHOR CONTRIBUTIONS

Z.H. and B.K.K. conceptualized and led the review. D.B.L.T. and L.D. performed the literature review, summarized data, constructed the figures and wrote the first version of the manuscript. B.K.K. and Z.H. revised and critically reviewed the subsequent draft revisions. All authors read, edited, and approved the final article.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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