MINIREVIEW

Ovarian Aging and Menopause: Current Theories, Hypotheses, and Research Models

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Aging of the reproductive system has been studied in numerous vertebrate species. Although there are wide variations in reproductive strategies and hormone cycle components, many of the fundamental changes that occur during aging are similar. Evolutionary hypotheses attempt to explain why menopause occurs, whereas cellular hypotheses attempt to explain how it occurs. It is commonly believed that a disruption in the hypothalamic-pituitary-gonadal axis is responsible for the onset of menopause. Data exist to demonstrate that the first signs of menopause occur at the level of the brain or the ovary. Thus, finding an appropriate and representative animal model is especially important for the advancement of menopause research. In primates, there is a gradual decline in the function of the hypothalamic-pituitary-gonadal (HPG) axis ultimately resulting in irregularities in menstrual cycles and increasingly sporadic incidence of ovulation. Rodents also exhibit a progressive deterioration in HPG axis function; however, they also experience a period of constant estrus accompanied by intermittent ovulations, reduced progesterone levels, and elevated circulating estradiol levels. It is remarkable to observe that females of other classes also demonstrate deterioration in HPG axis function and ovarian failure. Comparisons of aging in various taxa provide insight into fundamental biological mechanisms of aging that could underlie reproductive decline. Exp Biol Med 230:818–828, 2005

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Introduction

The average age of menopause in women is approximately 51 years, resulting in a postreproductive period that extends for nearly one third of their lives (1). Further, because many women have elected to delay reproduction, due to career and other considerations, they have encountered the reality of reproductive aging and ovarian aging. Consequently, the mechanisms involved in the process of ovarian aging has gained increased visibility and relevance. If menopause merely affected fertility, the study of ovarian aging would likely not be of such high priority. A number of other physiological systems are also affected by the sudden withdrawal of hormonal support associated with menopause, including: bone density, cardiovascular health, cognition and possibly some cancers (2–5).

Historically, it has been believed that female vertebrates acquire 100% of their primordial follicles at or around the time of birth. This doctrine has recently been brought into question by Johnson *et al.* (6), who demonstrated that ovarian follicular renewal may be possible in mice. Regardless of the process, however, by menopause the ovary is almost completely devoid of follicles. Throughout the reproductive life span of any female, the number of follicles that become atretic is much greater than the number of follicles that actually proceed through to ovulation. Thus,

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ovarian aging in one form or another is a consistent theme. There are subtle differences, of course, between species, challenging researchers to find appropriate models for studying female reproduction, especially as it relates to women.

The function of the hypothalamic-pituitary-gonadal (HPG) axis retains remarkable similarity across taxa. A period of reproductive immaturity is followed by a period of reproductive maturity and activity, finally ending with a period (variable in length) of reproductive senescence and loss of function. Reproductive decline during aging is evident in nonhuman primates, domestic livestock, elephants, whales, and lions as just a few examples (7). Evolutionary hypotheses seek to answer the question of why menopause occurs; cellular hypotheses attempt to answer the question of how it occurs; and research models create opportunities to study these questions in the laboratory.

Current Hypotheses on Female Reproductive Aging

Evolutionary Hypotheses and Theories. In humans, women experience a dramatic loss in ovarian function and subsequent menopause around the age of 50 (1). Thus, with the average life span ~70–80 years, a woman will spend a significant portion of her life in a postreproductive state. It has been suggested that menopause is simply a nonadaptive by-product of increased longevity in humans because it seems contrary to maximizing Darwinian fitness (8). That is, rapid advances in medicine, nutrition, and health have allowed people to live much longer than they have in the past. One hundred years ago the average life span was about ~30–40 years, so there was likely little to no postreproductive life. Some scientists believe that not enough time has passed for an evolutionary effect; therefore, menopause is little more than a side effect of increased life span. Others have hypothesized that menopause evolved as "quality versus quantity" trade off. Table 1 summarizes the following hypotheses and theories. Specifically, menopause may have evolved as a result of the rapid encephalization of humans in combination with acquisition of bipedal movement (9, 10). As humans became more adept at making and handling tools, brain size increased dramatically. Increased brain size resulted in a more altricial infant (helpless, with an extended parental care requirement). Additionally, evolution of bipedal locomotion resulted in pelvic alterations that made delivery more dangerous and difficult. It may be that as women aged, the mortality risk and energetic requirements of pregnancy, delivery, and lactation became too great to risk. The extreme altriciality of human infants requires a large parental investment. Thus, the Good Mother Hypothesis (11–13), states that there is a trade-off between increasing allelic contribution to the population (having more children) and ensuring the survival of present children. The Grandmother Hypothesis (8, 14, 15) is an extension of this theory, but it more directly addresses the postmenopausal period. It agrees with the general risk of pregnancy at advanced ages, but also addresses the contribution of the grandmother to her grandchildren in assuring her own personal "fitness." That is, a reallocation of her reproductive effort to cared-for children already born, as opposed to production of offspring unlikely to survive maternal death. Although we usually think of this reproductive strategy in the context of humans and possibly nonhuman primates, elephants and pilot whales also show this type of social structure and have an extended period of postreproductive life span. It is plausible that in some higher vertebrates, this strategy is useful in species that require extensive caretaking and energy investment during infant and maturing phases in the life span.

Table 1. Summary of Evolutionary Hypotheses and Theories of Menopause

Evolutionary hypothesis or theory	Description
Good Mother Hypothesis	Risk of pregnancy resulting in a trade-off between an increased number of children and ability to successfully raise them (11–13)
Grandmother Hypothesis	Reallocation of reproductive effort from production of own offspring to caring for offspring already born or children's offspring (9, 14, 15)
Disposable Soma Theory	Favoring of reproductive systems over maintenance and repair systems; menopause phenotype appearing with increased life span (16)

In a more general sense, the Disposable Soma Theory of Aging (16) is based on the supposition that organisms favor reproductive systems over systems responsible for maintenance and repair. In other words, an animal will place the majority of its energy into reproductive development, and maintenance and aging occurs as a result of "neglected" systems. An example of this theory may be found in the reproductive history of some laboratory mice that exhibit a systematic decline in function over time. In the wild, however, the mouse's reproductive ability may be perfectly functional up until its demise. The main difference, of course, is that in the laboratory, the mouse is in a protected environment and may live well beyond the age of a mouse in the wild. Thus, the Disposable Soma Theory states that the mouse has devoted most of its energy into maintaining reproductive function but would need to continue only as long as the animal is likely to survive. Following cessation of reproduction the mouse deteriorates physiologically, as little energy has been delegated into maintenance of its somatic functions, ultimately resulting in many of the aging phenotypes observed in captivity. Finally, it is clear in some species, such as mice, that animals showing an age-related deterioration in their "fitness" are likely to be predated; whereas, animals in the laboratory are likely to survive much longer. This suggests that the aging phenotype

observed in the laboratory would be rare in the wild, further supporting the validity of the Disposable Soma Theory. Like a protected captive animal, advances in human health and medicine have protected women and the rate of increase in life span may have exceeded the rate of increase in reproductive life span.

Cellular Hypotheses. *Ovary-Driven Reproductive Aging.* The depletion of follicular reserve and subsequent loss of fertility provide one explanation for how menopause occurs (17). The final population of oocytes in the adult female has historically been believed to be established during a stage in embryogenesis in which the primordial germs cells undergo a multitude of mitotic divisions and formation of oogonia (18). Cells enter meiotic prophase and remain arrested in the cell cycle as primary oocytes until puberty and exposure to appropriate levels of gonadotropins. During the peripubertal phase, development of gonadotropin-dependent granulosa cells mediate oocyte growth. Depending on the species, some preantral follicles are recruited, and one or more antral follicles are selected for ovulation. At this point, the recruited oocyte awaits signals from the pituitary for ovulation and resumption of meiosis. Follicular cells in the recruited oocytes undergo functional changes resulting in production of progesterone and preparation for fertilization. The prolonged cell cycle arrest just described is a unique feature of female vertebrate gonadal development. Thus, the follicular or ovarian reserve provides an exhaustible resource of oocytes and follicles that is established at or around the time of birth. Faddy *et al.* (19) reported the age-related biexponential decline in follicles. There is debate, however, regarding the accuracy of those data. Arguments suggest the biexponential decline is an artifact of the log-linear transformation and that follicle depletion is in fact monophasic (20). In any event, the ovarian follicular reserve declines during aging and the majority of follicles are lost in atretic processes (19, 21). It becomes very interesting to examine not only the fundamental biology of this process but also the potential for extending ovarian function with interventions known to affect overall life span.

Inhibin (INH) was identified in 1932 and shown to be involved in the regulation of the pituitary gland. It is a dimeric, glycoprotein hormone consisting of an α subunit with either β_A or β_B subunit, denoted as inhibin A (α-β_A; INHA) or inhibin B (α-β_B; INHB; Ref. 22). Using *in situ* hybridization, Roberts *et al.* (23) localized the expression of the inhibin subunits in human ovaries throughout the menstrual cycle. They determined that the α subunitwas expressed in the granulosa cells of small antral as well as in dominant follicles. Subsequent studies on circulating levels of inhibins throughout the menstrual cycles determined that INHB is the dominant inhibin produced in the early and midfollicular phase, whereas INHA is the dominant inhibin synthesized in the late follicular and luteal phases (24, 25). Interest in INH has become more prevalent with respect to reproductive aging and menopause as its association with follicle stimulating hormone (FSH) has become more defined. Increased levels of FSH in older women are evident throughout the cycle; however, this elevation is most consistent in the early follicular phase. It remains unclear whether rising FSH levels are directly due to lowered levels of inhibins. Soules *et al.* (22) hypothesized that once the number of preantral follicles falls below some critical threshold, the subsequent drop in INHB may result in rising levels of FSH (Fig. 1). Welt *et al.* (26) compared daily menstrual cycle hormone levels in younger and older cycling women to characterize the relationship between the inhibins and the menopause-associated rise in FSH. They determined that INHB remained lower among older cycling women throughout the menstrual cycle, whereas estradiol-17β (E_2) and FSH in the older women varied (either higher or lower than the young at specific times) when compared with FSH levels in younger cycling women. Therefore, FSH did not appear to be consistently predictive of an individual's reproductive system status. Overall, these data confirmed an inverse relationship between INHB and FSH as well as a general decline in INHB, INHA, and progesterone (P4) before detectable differences in circulating estradiol levels. The decrease in INHB appears to be one of the earliest hormonal events that may lead to the age-associated increase in FSH (26, 27). This lends credence to the hypothesis that reduced follicle numbers and consequently reduced INHB levels, lead to the monotropic rise in FSH observed in perimenopausal women.

Increased levels of FSH during reproductive aging have been documented across several species. de Souza *et al.* (28) demonstrated that endocrine changes occur with ovarian

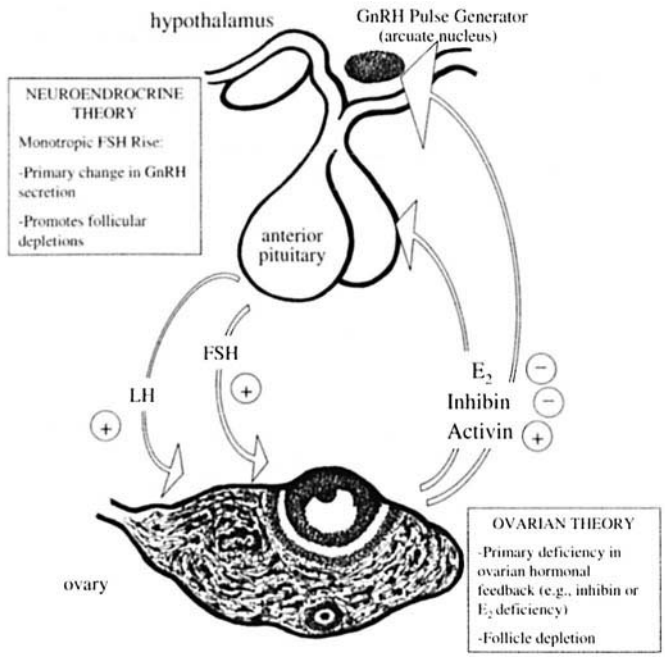

Figure 1. Aging and the female reproductive system. Reprinted from Figure 2 in Maturitas 30:193–204 by Soules MR, Battaglia DE, Klein NA; "Inhibin and reproductive aging in women," copyrighted 1998 by Elsevier Ireland Ltd. (22) Redrawn by Kimberly A. Ottinger.

aging in sheep and that the pattern of change was similar to that observed in women. Specifically, there were significantly fewer antral follicles available for development in older ewes during the early luteal phase, despite similar ovulation rates compared with their younger counterparts. Consequently, INHA was reduced in both the follicular and luteal phases in older animals, and this was associated with increased concentrations of FSH during the luteal phase. Estradiol levels, however, were similar between the two age groups. These data are consistent with the view that there is an overall depletion of the follicular pool during the process of aging. The maintenance of a similar number of ovulations in older animals suggests that a greater proportion of the follicular pool in an aging female is promoted through to ovulation. At some point, there appears to be insufficient number of developing follicles to produce the hormonal support necessary to stimulate hypothalamic gonadotropin-releasing hormone (GnRH) and the subsequent preovulatory luteinizing hormone (LH) surge. This diminished responsiveness and coincident decline in ovarian function snowball, leading to eventual reproductive failure.

Brain-Driven Reproductive Aging. There is evidence suggesting that the age-related increase in FSH levels ultimately leads to an accelerated follicular loss and subsequent ovarian failure. Changes that occur in the temporal pattern and synchrony of neurochemical and neuroendocrine signals may trigger the cascade of perimenopausal events. It has been suggested that miscommunication between the brain and the pituitary-gonadal axis occurs as a result of dampening and desynchronization of neural signals (29).

Meredith *et al.* (30) researched the effect of unilateral ovariectomy (ULO) on the rate of loss of primordial follicles. Young and middle-aged (MA) rats were ovariectomized unilaterally and observed for changes in the loss of primordial follicles. The investigators determined that ULO triggered an increased loss of follicles but only in the old rats. A subsequent study by Anzalone *et al.* (31) showed that ULO reduced follicular reserve in young and old rats. The effects of ULO were then compared in young virgin and MA breeder female rats relative to sham-operated controls. Results revealed that ULO reduced ovarian follicular reserve to levels similar to MA control rats. Although preovulatory estradiol-17β (E_2) levels were found to be similar between groups, there was reduced follicular reserve and significantly lower amplitude in the LH surge on the evening of proestrous. Moreover, the reduction in the LH surge was correlated with the numbers of resting follicles. Interestingly, despite similar follicular reserves in the young ULO and MA control rats, MA rats still showed lower peak LH levels as well as fewer regular cycles. Young ULO animals also had increased FSH levels on the morning of estrous (likely a compensatory response), whereas MA ULO rats did not. This elevated FSH release in young ULO rats was associated with an increase in follicular development so that the number of preovulatory follicles in the single ovary of these animals was similar to the number of preovulatory follicles in both ovaries of the young controls. Therefore, experimental reduction in ovarian reserve affected the LH surge, ovulation, and cyclicity in both young and MA ULO animals. However, young ULO females demonstrated a compensatory response by raising FSH levels despite reduced follicular reserve, suggesting a fundamental age-related alteration in the regulation of FSH unrelated to follicular reserve.

Limited data are available suggesting an age-related alteration in the central LH surge mechanism in perimenopausal women (32). Estradiol challenge in young and perimenopausal women showed the predicted transient decrease in LH and FSH levels, presumably associated with negative feedback at the level of the hypothalamus. Following this initial decrease, however, seven out of nine of the young participants exhibited a LH surge, whereas a similar surge occurred in only one of the eight perimenopausal women. Therefore, it appears that the perimenopausal HPG axis may have changes that involve more than subnormal ovarian function.

Isolating age-related changes in hypothalamic response from ovarian aging is difficult; however, a number of studies have been conducted using the rodent model. These studies have examined the response of the HPG axis at various stages in the life cycle of the female as well as the functional changes of the hypothalamus and pituitary gland with aging. Wise (33) reported decreased amplitude and frequency in LH secretion in older rats and a decline in the number of activated GnRH neurons, despite normal cycles (34, 35). The article by Wise (33) and many other articles point to alterations in the systems that modulate GnRH as key elements of the reproductive decline. Overall, these studies indicate that hypothalamic response decreases, potentially in tandem with declining ovarian function (33, 36, 37).

Comparative Models of Menopause and Ovarian Aging

Finding appropriate animal models for studying menopause is an often debated subject with many aspects. The clinical definition of menopause is the cessation of spontaneous menstrual cycling for at least 1 year. Primates are unique in the animal kingdom with regard to the extensive menstrual sloughing of the endometrial lining. Thus, in the strictest sense of the word, menopause research is only possible in animals that experience menses. Human and even nonhuman primate research can be prohibitive because of cost and complex variables in lifestyle, history, and time required to study aging systems. Therefore, there is a great need for alternative models of menopause. The ideal menopause model would have similarity to endocrine and neuroendocrine aspects of human ovarian biology. Additionally, if possible, the model should have reproductive traits that can be manipulated in the laboratory, that is, transgene or natural mutations resulting in accelerated or

Table 2. Summary of Research Models of Ovarian Aging and Menopause

Animal	Model	Phenotype
Bird	Chicken, etc	Normal ovarian senescence: varies by species (38–41)
Rodent	Mouse/rat	Normal ovarian senescence: ~6–18 months (17, 42, 43)
	Calorie-restricted rat	Delayed ovarian senescence (44–46)
	Bax$^{-/-}$ mouse	Delayed ovarian senescence (48–55)
	VCD[a]-treated mouse/rat	Accelerated ovarian senescence (58–65)
	*FoxO3*a$^{-/-}$ mouse	Accelerated ovarian senescence (66–72)
	FSH-R$^{+/-}$ mouse	Accelerated ovarian senescence (73–81)
Primate	Human	Normal ovarian senescence: ~50 years (1, 82–88)
	Rhesus monkey	Normal ovarian senescence: ~24 years (89–91)

[a] VCD, 4-vinylcyclohexene diepoxide.

delayed example of ovarian aging. Therefore, it is important to be able to compare normal aging to accelerated and delayed models as a way of elucidating the process as well as the underlying mechanisms contributing to the process of aging. This section will present some of the current research models that have been developed and are being used to study menopause and ovarian aging (see Table 2).

Avian Models. Vertebrate physiology can vary greatly, however, there are some conserved mechanisms that emerge relative to ovarian aging. There has been recent interest in the utility of other classes of vertebrates for understanding the reproductive, as well as the fundamental biology of aging (38, 39). Despite an apparent structural dissimilarity between the avian and mammalian ovary, there are a number of functional similarities as well. In most birds, only the left ovary develops, and the right ovary and oviduct regress perinatally. The ovary is grape-like in appearance because the deposition of yolk and follicles are arranged in a hierarchy that can be considered analogous to mammalian antral follicles (40). The appearance of the avian ovary is also remarkable because of the deposition of yolk into the larger cohort of follicles. The largest follicle is the first in line for ovulation, the second largest follicle is second in line for ovulation, the third largest follicle is third in line for ovulation, and so forth (41). Prehierarchical follicles that are in early stages of recruitment vary in appearance depending on the yolk deposition and include small, yolk-filled follicles (SYF); large, white, yolk-filled follicles; or small, white, yolk-filled follicles (39). These smaller prehierarchical follicles can be considered analogous to small antral follicles. Other areas of the reproductively active ovary contain primary and primordial follicles at various stages of development, similar to the mammalian ovary. Furthermore, as in mammals, the primary gonadal steroids are estradiol and progesterone, with other peptide hormones of lesser biological activity also produced. Therefore, there are similarities in the functional components of the avian ovary that are analogous to constituents of the mammalian ovary.

That stated, there are distinct differences in the operation and organization of the ovulatory cycle in avian females compared with mammals. Most notable is the lack of a distinct luteal phase (and absence of a uterus). This difference, however, provides one of the most distinct advantages for studying female reproduction in birds: ovulation events may be positively and noninvasively monitored by egg laying. Similar age-related changes in hypothalamic response, diminishing LH surge, and failing ovarian function have been found in some birds; however, the ultimate question of whether old hens demonstrate similar aging hormone profiles to women still remains unanswered. Of particular interest would be to investigate the presence or absence of the monotropic rise in FSH typically observed in human menopause. Some simple experiment paralleling in the ovarian aging phenotype between birds and mammals would help to substantiate the utility of the bird model in ovarian aging research.

Rodent Models. *Rodent Ovarian Biology.* Most rats will become reproductively mature at approximately 5 weeks of age. At reproductive maturity, they have an estrous cycle that lasts 4–5 days regardless of seasonal changes (17). Both rats and mice will begin to exhibit periods of persistent estrous, which is associated with elevated, constant levels of estradiol, low levels of progesterone, and a lack of LH surges and ovulations (17). The tonic levels of estradiol lead to stimulation and cornification of the vaginal epithelium, resulting in a state of persistent vaginal cornification. Laboratory mouse and rat strains differ with regard to age of ovarian decline both within and between species and may occur from 6 to 18 months of age, depending on strain (42). Ultimately, mice and rats will enter a final stage characterized by low plasma estradiol and progesterone levels, as well as little to no remaining developing ovarian follicles (43).

The use of rodent models in ovarian aging research is manifold. They are relatively short-lived, and the availability of homogenous laboratory strains permit controlled research experiments. As such, ovarian function in rodents is well characterized. Furthermore, genetic manipulation of specific genes has made it possible to study aspects of ovarian decline in transgenic models as well.

Delayed Ovarian Aging. Calorie-restricted rodent. Extension of overall life span has been well characterized in the rodent, using calorie-restriction protocols. Although the exact mechanisms of action explaining

how this occurs is still largely unknown, it is believed to act by altering or improving the function of a variety of physiological systems. Reproductive studies in calorie-restricted rodents have also been performed. Rats maintained at 50% body weight (as compared with control littermates) still achieved sexual maturation, albeit delayed (44). Onset of puberty in calorie-restricted rats was observed once animals reached body weights similar to pubertal controls (45). The onset of reproductive decline, however, was significantly delayed as well (44, 45). Nelson *et al.* (46) evaluated ovarian reserve in calorie-restricted (alternate day fasting) mice. The investigators found that in mice, calorie restriction suppressed estrous cyclicity, and a return to *ad libitum* feeding restored cyclicity. Histological data demonstrated that calorie-restricted rats had twice the number of primordial follicles as their age-matched controls. Furthermore, calorie-restricted mice maintained cyclicity at an age when their age-matched controls were 80% acyclic. Therefore, calorie restriction may delay reproductive senescence either by delaying puberty or initiating a period of ovarian "rest."

More recent data suggest that caloric restriction affects reproductive longevity at the level of the hypothalamus or pituitary (47). Investigators have found that female rats restricted to 60% of ad libitum feeding after the onset of puberty did not experience any interruption of normal cycling, but delayed cessation of estrous cycles was still observed. Therefore, they concluded that caloric restriction affected the reproductive system in rats by a mechanism other than simply delaying puberty or disrupting normal cycling. McShane and Wise (47) hypothesize that caloric restriction may actually preserve the reproductive neuroendocrine axis, allowing for prolonged reproductive ability in these animals. In control animals, LH concentration and pulse amplitude decline with age; however, calorie-restricted animals demonstrated enhanced LH secretion, and this may be attributable to some enhanced pituitary or hypothalamic factors. It was suggested that neuropeptide Y could be involved in such effects because it has been shown to increase during periods of food restriction.

Bax*$^{-/-}$ *mouse. The exact mechanism by which ovarian reserve depletion occurs is unknown; although, atresia and apoptosis are certainly involved. Bax is a member of the Bcl-2 family of proteins, which are considered to be pivotal in the regulation of cell-death pathways (48). The Bcl-2 family members are generally classified as either proapoptotic, such as Bax, Bid, and Bad, or antiapoptotic, such as Bcl-2 and Bcl-X_L (48). These proteins are believed to exert their apoptotic effects via hetero- and homo-dimerization (49–51) and the ability to form membrane channels, thereby, influencing ion or protein transport (52, 53).

Bax has been localized in both granulosa cells and oocytes (54–56). Delayed ovarian aging has been documented in a knock-out mouse model: the *Bax*$^{-/-}$ mouse (56). On gross examinations, 20–22-months-aged *Bax*-deficient female mice exhibited uterine hypertrophy as compared with their age-matched controls. Morphological analyses revealed the presence of multiple follicles at varying stages of development, including large antral follicles with visible oocytes. No indication of ovulation (presence of corpora lutea) was noted; however, retrieval of oocytes following a superovulation protocol indicated that a mixture of normal, mature, and abnormal oocytes were present in these animals. As expected, the age-matched control ovaries chiefly consisted of stromal tissue, lacking evidence of follicles or oocytes.

Investigators (57) performed morphometric analyses to determine if the sustained follicle endowment observed in *Bax*-deficient female mice was attributable to a greater initial ovarian follicle reserve. Neonatal wild-type and *Bax*$^{-/-}$ mice were found to have similar numbers of nonatretic primordial and primary follicles. Shortly after puberty, however, *Bax*$^{-/-}$ mice exhibited three times the ovarian reserve of their wild type counterparts. The authors hypothesized that the *Bax* deficiency may have granted some protection to the granulosa cells and oocytes against apoptosis. This model provides a unique perspective by delaying, if not eliminating, ovarian senescence in the mouse. *Bax* levels have been shown to be elevated with the initiation of cell death in the human ovary (55). Thus, the *Bax*-deficient female mouse model is a useful and intriguing model for studying menopause and the decline in the ovarian follicular reserve in women.

Accelerated Ovarian Aging. VCD-treated rodent. 4-Vinylcyclohexene diepoxide (VCD) is an industrial chemical that is made during the production and manufacture of insecticides, plasticizers, antioxidants, flame retardants, and rubber tires (58). It is being studied with regard to its ovotoxic effects and risk factor for premature menopause in women; however, it also provides an interesting model for accelerated ovarian aging (58, 59). VCD will selectively destroy primordial and primary follicles when administered repeatedly to mice and rats (60). The exact mechanisms by which the ovotoxicant VCD acts to initiate atresia are still largely unknown; however, it is believed to act by accelerating the natural processes of atresia (60, 61). Induction of premature ovarian failure is thus possible via depletion of the pool of primordial follicles. The mechanism of action by which VCD acts is thought to be via proapoptotic pathways (62).

After 30 days of treatments (80 mg/kg VCD per day), Fischer-344 rats had significantly reduced numbers of preantral follicles (63). There were no apparent ultrastructural difference between groups; however, circulating levels of FSH were elevated by 120 days in VCD-treated rats as compared with vehicle animals. Furthermore, cyclicity was disrupted in VCD-treated rats by 360 days (63). Experiments in mice yielded similar results: VCD-treated mice exhibited elevated levels of circulating FSH and reduced estradiol at an age where control hormone levels were still normal (64). Secondly, androgen levels in VCD-treated mice have also been shown to be elevated,

similar to postmenopausal hyperandrogenic women (65). Combined, these data demonstrate that the accelerated time frame of the onset of ovarian senescence in the VCD-treated mouse supports its use as a menopause model, particularly of premature menopause. Moreover, this model may provide insight into the apoptosis-regulated pathways that likely lead to ovarian depletion and, consequently, menopause in women, whether normal or premature.

Foxo3a$^{-/-}$ mouse. Foxo3a is a member of the mammalian FOXO subfamily of forkhead transcription factors, including Foxo1, Foxo4, and Foxo6 (66). FOXO transcription factors may be considered analogous to the DAF-16 transcription factor (and thus part of the DAF-2 pathway) in the roundworm (*Caenorhabditis elegans*; Ref. 66). This is of considerable interest given exciting research that has shown that loss-of-function mutations in DAF-2 extends life span in the roundworm (67, 68). The role of *Foxo* genes in mice is currently being explored; however, it is clear that the individual genes (*Foxo*1, *Foxo*3a, and *Foxo*4) are functionally diverse (69). *In vitro* data suggest a role for *Foxo* genes in cell cycle arrest, apoptosis, and specific stress responses (70, 71).

More specifically, *Foxo*3a has been found to be an essential regulator and suppressor of follicular activation (72). Investigators (72) generated a *Foxo*3a$^{-/-}$ mouse bearing a null mutation in the *Foxo*3a locus. These mice appeared outwardly normal up to 48 weeks of age, with no differences in body weight or increases in cancer or mortality. With regard to reproduction, however, Castrillon *et al.* (72) reported sterility in these mice by 15 weeks of ages, despite normal sexual maturation (based on first litter). Histological analyses of ovaries indicated normal ovaries at postnatal Day 3 (PD3), but by PD8, *Foxo*3a$^{-/-}$ ovaries were consistently enlarged. These mice exhibited early follicular activation, maturation, and atresia, consequently resulting in early depletion of ovarian reserve. By 20 weeks, *Foxo*3a$^{-/-}$ females demonstrated classic signs of hypogonadotropic (elevated FSH and LH) hypogonadism, typical of premature ovarian failure. The authors suggest that these results indicate a role for *Foxo*3a specifically in follicular growth but not other aspects of follicular maturation and reproduction. Thus, these data suggest that accelerated follicular initiation may be an underlying cause for premature ovarian failure.

FSH-R$^{+/-}$ mouse. As its name implies, FSH is the main hormone responsible for the processes involved in folliculogenesis, including follicular growth and differentiation (73). FSH receptors are found exclusively on the granulosa cells in the ovary and the Sertoli cells in the testis; therefore, all of the hormone's actions occur in these tissues (74, 75). FSH is essential for follicular maturation and the synthesis of estradiol from the granulosa cells via theca-derived androgen aromatization (73). In addition, rats treated with FSH (10 μg/injection, twice daily) have shown a decrease in DNA fragmentation and apoptosis in the ovary, suggesting a protective effect of the gonadotropin (76). Furthermore, some investigators have proposed the idea that ovarian follicles in aging females may become refractory to gonadotropin stimulation, impairing ovarian response and function (77). These data, along with previously cited data reporting the elevated FSH levels coincident with ovarian decline, underline the need to elucidate the role of the FSH receptor in age-related ovarian failure.

The FSH-receptor haploinsufficient mouse (FSH-R$^{+/-}$) has been proposed as a model for studying menopause (78). The follitropin receptor knockout (FORKO) mouse was first characterized by Dierich *et al.* (79). Female FORKO (FSH-R$^{-/-}$) mice are infertile, and their ovaries are significantly smaller than their wild-type littermates because of a lack of large Graafian follicles and the absence of corpora lutea (77). Conversely, FSH-R haploinsufficient mice did reach reproductive maturity, and that maturity occurred earlier than in their wild-type counterparts (80). Litter sizes in the FSH-R$^{+/-}$ mice were also lower at all observed ages (3, 7, and 12 months). Gross examination of ovarian histology in 3-month-old mice showed little differences between wild-type and FSH-R$^{+/-}$; however, closer inspection revealed evidence of an increased numbers of atretic follicles in the FSH-R$^{+/-}$ animals. By 7 months, there was an increase in the numbers of abnormal-looking follicles, with irregular-looking or double oocytes. Additionally, although there was no difference in the total number of follicles in 3 month FSH-R$^{+/-}$ versus wild types, 7-month FSH-R$^{+/-}$ ovaries had significantly reduced numbers of oocytes as compared with their age-matched controls. The accelerated loss of oocytes may be attributable to increase cell death because the percentage of atretic follicles was much increased in the FSH-R$^{+/-}$ animals, at all ages (80).

Danilovich *et al.* (81) have also reported on the endocrine alterations in the FSH-R$^{+/-}$ haploinsufficient mouse. Young FSH-R$^{+/-}$ mice (3 months) have reduced estradiol but similar pituitary gonadotropin levels to their wild-type counterparts. By 7 months, however, FSH-R$^{+/-}$ mice exhibit elevated gonadotropins and reduced estradiol levels (coincident with reduced numbers of ovarian follicles). These data indicate that the FSH-R$^{+/-}$ mouse experiences accelerated ovarian aging and may be a useful model for studying menopause (81). Furthermore, the authors suggest that these studies support the hypothesis that declining FSH-R and increasing FSH levels in aging females is a result of increased ovarian resistance to follicular development (81).

Primate Models. *Human Ovarian Biology.* In the United States, menarche occurs in young women at an average age of 12.5 years (82). Menstruation is unique to primates and occurs as a result of sloughing of the endometrial lining. The menstrual cycle consists of the follicular phase and the luteal phase, divided by ovulation. Each phase lasts approximately 14 days, resulting in an median menstrual cycle length of 28 days (83). Clinical menopause is defined as the period of a woman's life 1 year

after the cessation of menstrual cycles occurring at a media age of 51 years (1). The events leading to the climacteric begin years earlier and are termed the perimenopausal transition. Data collected by the Center for Disease Control (CDC) have shown that there is a significant decrease in the incidence of pregnancy and an increase in spontaneous miscarriage, ectopic pregnancies, and chromosomal abnormalities even in young women (~30 years; Ref. 84).

The monotropic rise in FSH is the hallmark event indicating the onset of reproductive decline in women, showing significant increases in women before menstrual irregularity or other endocrine changes (85). Increased levels of FSH are evident throughout the cycle; however, the difference is most consistent in the early follicular phase. FSH levels on menstrual cycle days 2–5 have been shown to correlate well with ovarian reserve and are commonly used in clinical practices to evaluate and predict pregnancy success via artificial reproductive technologies (ARTs; Ref. 86).

Use of human subjects for investigating reproductive senescence permits the most direct application of research into practice. Fortunately, the increased demand for human ART has supported the rapid advancement of research in human reproduction. Unfortunately, some of this demand may be because of the trend toward postponement of childbearing and the increased incidence of infertility with age (29). Aging in the ovary appears to play a more important role in declining fertility than uterine aging (4). Navot *et al.* (86) demonstrated that when age of the oocyte donor was controlled, there were no significant differences in the delivery rate between young and old recipients. Within older cohorts, however, there may be differential fertility; thus, ovarian age is not necessarily dictated by chronological age (87). Furthermore, ovarian age may be determined by ovarian reserve, and this is dependent on the pool of remaining follicles as well as the quality of the oocytes.

DNA microarray analyses of luteinizing granulosa cells by Chin *et al.* (88) demonstrated differences in gene expression between women with normal or decreased ovarian reserve. Diminished ovarian reserve was determined based on Day 3 FSH and peak serum E_2 levels and number of oocytes retrieved following an ovarian stimulation protocol. The authors admit the difficulty in interpreting these data, given small sample sizes, differential responses within groups, and inconsistent gonadotropin stimulation of the subjects. It is still of great interest, however, to note that there were changes in a few specific genes. Although inconclusive, these data provide a basis for identifying specific gene targets that vary within the ovary during aging, which will be useful for future research on ovarian aging and menopause in women.

Rhesus Monkey Ovarian Biology. The rhesus monkey *(Macaca mulatta)* has been considered a useful model for reproductive studies in women since the early 1900s (89). Female rhesus monkeys are pubertal by 2.5–3.5 years of age and exhibit menstrual cycles approximately 28 days in length, similar to women. Hormonal and menstrual similarities to women have made the rhesus monkey a favored model in which to research ovarian function. Furthermore, rhesus monkeys experience a reproductive decline much like that of human menopause at approximately 24 years (90). One major difference in the menstrual cycle of humans versus rhesus monkeys, however, is the existence of a breeding season. Both indoor- and outdoor-housed rhesus monkeys experience a breeding season that varies between primate facilities but generally runs from September through May. Thus, further characterization of the onset of ovarian decline in rhesus monkeys was necessary to validate it utility as a model for human menopause.

The first report of a longitudinal assessment of menstrual patterns in aging rhesus monkeys was performed by Gilardi *et al.* (90). Gilardi *et al.* (90) reported urinary hormone profiles for 26 perimenopausal rhesus monkeys. Menstrual records were documented for 12 months before the urine collection period. Subsequently, daily urinary estrone conjugate (E_1C) and pregnanediol-3-glucuronide (PdG) were analyzed for 12 weeks in female rhesus monkeys aged 20.5–29.5 years (average 23.5). As expected, the younger monkeys (less than 25 years) menstruated regularly, whereas the older monkeys demonstrated an increasing menstrual irregularity. Similar to women who experience low estradiol and irregular progesterone profiles with the onset menopause, menopausal rhesus monkeys had low E_1C levels as well as irregular patterns of PdG. These data initially suggested parallel events in rhesus monkeys and women with regard to menopausal onset.

Further research by this group included analyzing urinary FSHβ and circulating inhibin B (INHB) in rhesus monkeys (91). As discussed earlier, elevated circulating FSH is the hallmark event associated with the onset of ovarian decline in women. Twenty female rhesus monkeys between 18 and 26 years were analyzed for menstrual cycling, circulating INHB (2 samples collected between menstrual cycle Days 3–5 and Days 10–12), as well as daily urinary E_1C, PdG, and FSHβ for 1 year. In agreement with the previous study, irregular menstrual cycling was associated with reduced urinary E_1C levels. Additionally, Shideler *et al.* (91) showed that urinary FSHβ levels were elevated in aged females. Unlike women, however, this elevation was only detectable after age-related menstrual irregularities were observed. Furthermore, decreases in circulating INHB levels were only detectable in ovariectomized monkeys as compared with normally cycling females. Therefore, the researchers acknowledge some key differences between the onset of menopause in rhesus monkeys and women.

Other Nonhuman Primates. Very little data exist characterizing menopause in other nonhuman primate species (92). Menopause is simply the cessation of menstrual cycling; thus, by definition it is a state that can

be achieved by any animal that experiences periodic endometrial sloughing and vaginal bleeding, including baboons, chimpanzees, gorillas, and orangutans (93–97). The baboon has been considered a useful model for various reproductive studies because of its similarities in menstrual cycle characteristics (98). Furthermore, the swelling of the perineal sex skin permits the noninvasive detection of the follicular phase and approximate time of ovulation (98, 99). Despite the use of the baboon in pregnancy and endometriosis research, however, very little had been reported on baboon menopause and the onset of reproductive decline. Menopause research in the great apes has been even less prevalent in the literature.

Summary

As advances in the biomedical field are made and increases in human longevity are observed, the issue of postmenopausal life span becomes increasingly important to understand. Consequent to the menopause-related hormone withdrawal are increases in cardiovascular, osteoporotic, cognitive, and cancerous diseases. Despite the encompassing effects menopause has on women's health, little is known about the triggers of this inevitable biological life stage.

Questions regarding how and why menopause occurs are inexorably linked, and much remains to be uncovered. Is menopause adaptive or nonadaptive? Is reproductive decline driven by the ovary or the brain? Certainly, depletion of the ovarian reserve seems to be central to this phenomenon. Conversely, hypothalamic and pituitary changes appear to play a major role as well. These questions are compelling and require the use of appropriate and available animal models. Each model will likely have advantages and disadvantages (whether monetary or biological), and these will need to be addressed on a case-by-case basis depending on the particular research question at hand.

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