Chapter 21

Aging of the Female Reproductive System

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I. Introduction

This chapter reviews the changes that occur in the female reproductive system with age. For most physiological processes, male organisms are studied more frequently than females in any area of biology. Thus, it is unusual that in terms of reproductive aging, more attention has been paid to females than to males. This is probably because (1) menopause occurs relatively early during the aging process so may serve as a model system in which to study the biology of aging of other systems, and (2) in humans and Old World monkeys, female reproductive aging is clearly punctuated by an easily observed endpoint: the cessation of menstrual bleeding. Therefore, it is relatively easy to document changes before and after this endpoint and, when desirable, to normalize the timing of specific changes relative to this endpoint. In males, changes are more gradual, and functional decline is not marked by an easily measurable endpoint; thus, it has been more difficult to document when the reproductive axis declines and when these changes have functional repercussions.

Emphasis will be placed on changes that occur in humans and animal models. The majority of basic science studies that have been performed using animal models have been performed in rats and mice, although some work has been done in nonhuman primates. Therefore, the majority of the work that will be reviewed in this chapter is from studies performed with these species.

II. Menopause

Although there is considerable variation in the exact age of menopause, the majority of women go through menopause at approximately 51 years of age (Treloar, 1981; Treloar *et al.*, 1967), and the timing of this change has remained essentially the same since medical records have been maintained. Menopause occurs around the time that the ovarian follicular reserve becomes exhausted, and, in fact, marked variation in the follicular

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reserve correlates with variation in deterioration of regular menstrual cyclicity. Because these cells are not only the source of germ cells but also are the key cells that produce and synthesize ovarian steroids and peptide hormones, plasma levels of all of these hormones drop dramatically after menopause and remain low for the remainder of a woman's life. In particular, the ovarian steroids, estrogens and progestins, decrease dramatically and remain low unless a woman chooses to take hormone therapy. During the past decade, investigators have realized that ovarian steroids are not only reproductive hormones, but that they are hormones that play roles in a wide variety of nonreproductive functions as disparate as bone and mineral metabolism, memory and cognition, cardiovascular function, and the immune system. Thus, the end of reproductive life and the cessation of synthesis and secretion of estrogens, progestins, and peptide hormones have far-reaching implications for the health and quality of life of women. With the dramatic increase in the average life span of humans from approximately 50 years to over 80 years, which has occurred during the last 100 years, and the relatively unchanging age of menopause, the number and the proportion of women who are destined to spend over one-third of their lives in the postmenopausal state with unique gender-related medical and social challenges has become substantial. It should not be surprising then that an increasing number of clinical and basic science studies have focused on understanding fully the physiological changes that accompany menopause, the mechanisms that drive reproductive aging, and the impact of these changes on women's health.

A better understanding of reproductive changes will be important to gerontologists because the female reproductive system deteriorates early during the aging process, in the absence of pathological changes that often confound gerontological studies. Therefore, we hope that the understanding and concepts derived from our deepening understanding of menopause and the aging reproductive system may apply more generally to the process of the biology of aging of other systems.

It is interesting to note that nonhuman primates exhibit a similar transition to acyclicity; however, in general, it occurs at a much later stage of the life span, and many nonhuman primate species do not have a prolonged postmenopausal period (Bellino, 2000). In fact, in some nonhuman primate species, reproductive cyclicity does not completely cease in some individuals before they die. Whether this is a fundamental difference between humans and nonhuman primates or whether this is the result of inadequate knowledge as to how to optimally maintain these species in "laboratory" environments is not clear. If it is the latter, we would predict that by improving their diets or health care, the way we have been able to intervene in human populations during the past 100 years, we will be able to prolong their average life spans. It will be interesting to observe whether menopause will then occur closer to the middle of this more prolonged life span.

There has been an ongoing and lively debate as to whether rodents serve as a good model for human reproductive aging. In some sense, because rodents do not undergo a true menstrual cycle—that is, there in no sloughing of the uterine wall at the end of each cycle and the resultant vaginal bleeding—rodents cannot undergo a true "meno-pause." In addition, in rats and mice, there is no true luteal phase of the estrous cycle because the corpus luteum regresses rapidly after ovulation and progesterone is not secreted for a prolonged period of time. Arguments that rodents are not good models center primarily around two

findings. First, in women, the loss of primordial follicles is log-linear during the initial stages of life and accelerates dramatically around the time women are 37 years old. This accelerated loss leads to the total absence of follicles when women are between 50 and 55 years old, when they are postmenopausal (Crowley *et al.*, 1985). In contrast, different strains and species of rodents exhibit striking variation in the rate of follicular loss. Although no studies have followed the rate of follicular loss across the entire life span, Faddy and colleagues (1987) showed that during the first 100 days of life, if anything, the rate of follicular loss decreases with age in mice. These data would suggest that, by the time rodents are reproductively senescent, the follicular pool may not be a limiting factor. Ovarian aging does play some role even in rodents since Gosden and colleagues (1983) showed a correlation between the size of the follicular reserve and entrance into irregular cyclicity. In addition, grafting young ovaries into mice about to become anovulatory extends their cycling life span (Felicio *et al.*, 1986). Second, in postmenopausal women, gonadotropins (hormones secreted from the anterior pituitary gland) concentrations in the circulation are elevated. It is thought that this is primarily in response to lowered estradiol secretion from the ovary because gonadotropin concentrations decrease in response to estrogen therapy. In contrast, gonadotropin concentrations remain relatively normal in old acyclic, repeatedly pseudopregnant rats. Thus, despite decreases in estradiol, gonadotropins do not appear to exhibit the dramatic increases observed in women. This suggests that decreased hypothalamic function leads to the post-reproductive state in rodents. The differences and similarities in reproductive aging between humans and rodent or nonhuman primate models are considered in detail later in this chapter.

III. Definitions

A. Terminology Used to Define Stages of Human Menopause

Investigators have used several terms to define different stages of the period of women's lives that surround the end of their reproductive life: perimenopause, climacteric, menopause, and postmenopause. *Perimenopause*, also called the *climacteric*, begins before menopause. This interval, which lasts approximately fours years, is the entire transition from the reproductive to the post-reproductive period of women's lives (Lobo, 1998; Prior, 1998). Symptoms such as hot flashes and irregular menstrual cycles may start to appear. By definition, perimenopause continues through the 12 months following the last menstrual period. Menopause is the permanent cessation of menstruation. Despite significant variation, menopause normally occurs spontaneously at approximately 51 years of age and is associated with the depletion of the ovarian follicular reserve. The term *surgical menopause* is defined as the cessation of menstrual cyclicity that results from the removal of a woman's ovaries when she would not normally undergo reproductive aging. Although both result in the cessation of menstrual cycles and the onset of infertility, the repercussions of natural menopause and surgical menopause may be different because the age of normal menopause is considerably greater than surgical menopause. Hence, changes in reproductive hormones in older women may result from interactions between chronological aging of the whole organism and aging of the reproductive system, whereas in younger women who are experiencing fewer age-related changes in other systems, the changes are likely to be related specifically to changes in the feed-forward and feed-back of the reproductive axis. Table 21.1 summarizes the definitions that are recommended by the World Health Organization.

Table 21.1
Definitions

Terminology	Definition
Menopause	Permanent cessation of menstruation associated with loss of ovarian follicular activity
Perimenopause or Climacteric	Period immediately prior to and at least one year after menopause, characterized by physiological and clinical features of altered ovarian function
Postmenopause	Period of life remaining after menopause
Premenopause	The reproductive period prior to menopause

B. "Menopause" in Species Other than Humans

Investigators once thought that menopause was restricted to human females. However, several papers (Gilardi *et al.*, 1997; Gould *et al.*, 1981; Graham *et al.*, 1979; Hodgen *et al.*, 1977) demonstrate that several species of nonhuman primates undergo a process very similar to that which women experience across menopause. However, these changes occur later in their average life span; therefore, the postmenopausal period is considerably shorter in these nonhuman primates species compared to women. At the present time, we do not know whether this is because menopause is truly delayed in nonhuman primates compared to humans or whether the nonhuman primate species that have been studied have not been maintained under optimal laboratory conditions and therefore their postmenopausal life span could be extended under different, more optimal environmental conditions. Currently, the populations of nonhuman primates that are available for study, which are maintained under controlled laboratory conditions, are small, and few studies have focused on aging. However, there are distinct advantages to using these species as models: longitudinal characterization from a population of animals that have been followed through their reproductive life span should be possible; intensive monitoring of hormonal changes through urinary samples is feasible; and records of reproductive history, in terms of numbers of pregnancies and live young, can be obtained. In addition, it is possible to perform longitudinal studies that are invasive and sometimes terminal in these species. Together, this means that use of these species in aging research will allow us to probe the underlying mechanisms that drive the menopausal transition. Such studies are expensive and labor-intensive; however, because an increasing number of nonhuman primate colonies have been maintained in captivity at several research centers, such studies will provide new data in the next several years.

Studies reveal several similarities in hormone profiles in older female rhesus monkeys and women during the transition from regular to irregular menstrual cycles. Variable inter-menstrual intervals and delay of ovulation interspersed with breakthrough uterine bleeding were found in perimenopausal rhesus monkeys (Gilardi *et al.*, 1997) and women in the fifth decade (Shideler *et al.*, 1989). However, it appears that not all of the hallmarks of human menopause punctuate the menopausal transition in the rhesus monkey. Importantly, in initial studies (Shideler *et al.*, 2001), the harbinger of impending reproductive decline, a selective rise in follicle stimulating hormone (FSH) concentrations in the absence of any change in luteinizing hormone (LH) levels, does not appear to occur in monkeys approaching

menopause prior to overt changes in menstrual cycle length. In addition, rhesus monkeys do not exhibit frequent periods of high, unopposed estrogen in association with the transition to the menopausal state as has been observed in women (Gilardi *et al.*, 1997; Santoro *et al.*, 1996). Studies are ongoing to determine whether the baboon may be a better nonhuman primate model of human menopause; however, aging colonies of this species are even less available than rhesus monkeys. It is clear from these initial tantalizing results that considerably more work is required before we will know which of these other species can be used to model human menopause.

IV. Role of the Ovary in Reproductive Aging

A. Depletion and Aging of the Oocyte Reserve

A large body of evidence suggests that, in women, exhaustion of ovarian follicular reserve is the major factor that underlies the timing of perimenopause and menopause. Thus, ultimately, the permanent cessation of menstrual cyclicity can be largely attributed to changes within the ovaries. It appears that females are born with an enormous, but finite, postmitotic, nonrenewable endowment of follicles. This follicular reserve is set down during fetal development: germ cells undergo mitosis for a time while they are outside the ovary proper and migrate into the undifferentiated gonad. They then cease replicating, initiate meiosis, organize into primordial follicles, and most remain dormant for many months to years. Once mitosis stops, no new germ cells will ever be added to the original reserve. This basic tenet of mammalian ovarian biology has been challenged recently in a study performed in mice (Johnson *et al.*, 2004). However, the methods used in this paper make it important to perform further experiments before a paradigm shift in our thinking is warranted. Within this follicular stockpile, a selected few will be recruited to undergo all of the steps that lead to the step of fertilization: growth, differentiation, and ovulation. The vast majority will re-awaken from the dormant pool and begin the path of growth and differentiation but will never undergo this entire process of development, differentiation, and maturation. Instead, they will undergo atresia through apoptic mechanisms of cell death before they are ever recruited to grow or at a step of the differentiation and maturation process (see Gougeon, 1996; Hirshfield, 1991 for reviews). Because atretic follicles cannot be replaced, the number of follicles continues an inexorable decline until relatively few, poorly responsive follicles remain at the time of menopause.

From fetal life through childhood into the reproductive years and ending at menopause, follicles reawaken and mature from primordial to secondary follicles. In postmenopausal women, the endowment of ovarian follicles is completely depleted (Block, 1952; Costoff & Mahesh, 1975;). In fact, Richardson and colleagues (1987) demonstrated that middle-aged women who had already begun menopausal transition and were exhibiting irregular menstrual cyclicity had 10 times fewer follicles in their ovaries than women who continued to cycle regularly. One of the most intriguing and provocative findings in the area of ovarian aging is that the rate of follicular loss is not log-linear (see Figure 21.1) (Gougeon *et al.*, 1994; Richardson *et al.*, 1987). Instead, the rate of follicular loss accelerates three- to six-fold when women are approximately 39 years of age. This change occurs at least 10 years prior to menopause and leads to complete depletion of the follicular endowment by the time women are in their fifties.

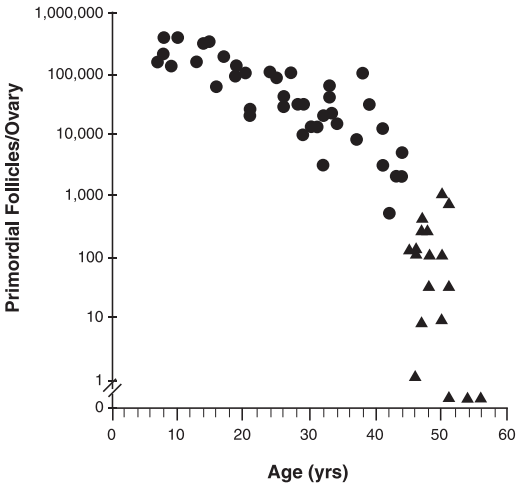

Figure 21.1 Age-related decrease in the total number of primordial follicles within both human ovaries from birth to menopause. As a result of recruitment, the number of follicles decreases in a log-linear fashion until approximately 37 years of age (circles), then the rate of decrease in the follicular pool accelerates (triangles) such that there are virtually no follicles left in the ovary at the time of menopause. From Richardson *et al.,* 1987; Copyright 1987, The Endocrine Society.

Gougeon and colleagues (1994) differentiated between the rate of disappearance of primordial non-growing follicles versus early growing follicles over the life span. Their results support the view that depletion of the pool of non-growing follicles is caused mainly by atresia of dormant primordial follicles in younger women, but mainly by the entrance of non-growing follicles into the growing pool in older women. If the rate of follicular loss did not change during this critical interval, the reserve of ovarian follicles would not be exhausted until women were between 70 and 80 years old. Thus, if the size of the follicular pool is the rate-limiting factor in female reproductive aging, the timing of reproductive senescence might not differ remarkably compared to the timing of decline of multiple other physiological systems. What leads to this accelerated loss of follicles during middle age? Unfortunately, few answers are available because the mechanisms leading to atresia within the non-growing dormant pool of primordial follicles and the regulation of the re-entry of resting follicles into the growing pool and their initial stages of growth are largely unknown, even in young females. The very early stages of follicular development appear to be independent of hormonal influences, such as gonadotropin levels or patterns of secretion or intraovarian concentrations of steroids or ovarian peptides. However, the final stages of follicular development and differentiation depend on hormone concentrations and their patterns of secretion, and only if the proper amount, proper patterns of secretion, and proper sequence of hormonal events occur do primordial follicles fully mature and ovulate. Because the primary factor that determines the rate of exhaustion is the rate at which dormant follicles re-awaken and move into the growing pool, this parameter is the critical factor—but probably the most difficult—to study. Some believe that the number of follicles in the primordial endowment is itself the major factor that determines the rate at which follicles begin to grow. According to this theory, and when the follicular reserve goes below a "threshold," regulation is compromised (Krarup *et al.*, 1969). Several studies support this view. Unilateral ovariectomy of older rats, or more drastic ovarian resection, accelerated the loss of the remaining primordial follicles (Meredith & Butcher, 1985; Meredith *et al.*, 1992). Meredith and colleagues (1992) found that unilateral ovariectomy only affected the rate of loss of follicles in older rats. In contrast, no difference was observed in numbers of follicles remaining in the single ovary or their rate of depletion in younger rats. In addition, destruction of a portion of the follicular stockpile by prenatal treatment with busulfan (Hirshfield, 1994) or postnatal treatment with xenobiotics (Krarup *et al.*, 1969), which caused

younger animals to have a follicular reserve that was more similar to middle-aged rats, also increased the rate at which the remaining primordial follicles moved into the growing pool. Taken together, these data suggest that a decrease in the number of primordial follicles results in an amplifying cascade that exacerbates the further loss of the follicular reserve.

Declining fertility and fecundity have been well documented prior to the exhaustion of the follicular pool (Santoro *et al.*, 2003). Evidence suggests that the predominant effect of age on fertility is due to abnormalities present in the older oocyte; however, changing uterine receptivity and embryo-uterine crosstalk certainly contribute to the lower rates of fertility and fecundity. Oocytes from normal younger and older reproductive aged women, examined at the second metaphase of meiosis, exhibited distinct structural differences and chromosomal abnormalities (Angell, 1994; Battaglia *et al.*, 1996; Battaglia *et al.*, 1997). The most compelling evidence for an effect of the aging oocyte on female fertility comes from clinical studies of donor oocyte in *in vitro* fertilization programs. Pregnancy and delivery rates are much more strongly correlated with age of the donor than age of the recipient or the age of the sperm donor (Klein & Soules, 1998).

B. Age-Related Changes in Ovarian Hormone Secretion

For many years, it was thought that decreased estradiol concentrations heralded the onset of the perimenopausal transition and that changes in pituitary gonadotropin levels followed as a result of decreased negative feedback. In fact, we now know that middle-aged women exhibit changes in FSH and inhibin before any obvious change in estradiol occurs (Klein *et al.*, 1996; Reame *et al.*, 1996). In fact, older ovulatory women who continue to have menstrual cycles of normal length show preovulatory urinary estrogen levels that are elevated and rise earlier in the menstrual cycle than younger women (Santoro *et al.*, 1996). Santoro and colleagues found that daily urinary steroid metabolites, estrone conjugates and pregnanediol glucuronide, were higher in premenopausal women 43 years old and older and compared these hormones to women between 19 and 38 years of age during both the follicular and luteal phase of the menstrual cycle (see Figure 21.2). Because FSH levels were also elevated in these women, the change in this hormone may have led to an earlier selection and development of the dominant follicle, which, in turn, could lead to relative increases in early follicular phase estradiol secretion (Klein *et al.*, 1996). It is interesting that elevated estradiol during the early follicular phase rise (i.e., day 3 of the menstrual cycle) is an excellent predictor of a poor response to treatments for infertility (Licciardi *et al.*, 1995). The pattern of age-related changes in estradiol in laboratory rats is strikingly similar to that reported in women: estradiol levels are essentially normal during the middle-age period of time when animals continue to cycle (Lu *et al.*, 1985; Nass *et al.*, 1984; Wise, 1982b;). Thus, in rats as in humans, it is not until the later stages of reproductive senescence that estradiol concentrations are clearly lower than those observed in young (Metcalf *et al.*, 1981; Santoro *et al.*, 1996).

One of the earliest detectable changes in ovarian hormone levels is a change in inhibin levels that are associated with a monotropic rise in FSH concentrations (Klein *et al.*, 1996; MacNaughton *et al.*, 1992) (see Figure 21.3). These data have led to the conclusion that the decrease in the number of primordial and early antral follicles remaining in the ovaries of older women leads to decreased inhibin B concentration. This small rise in the

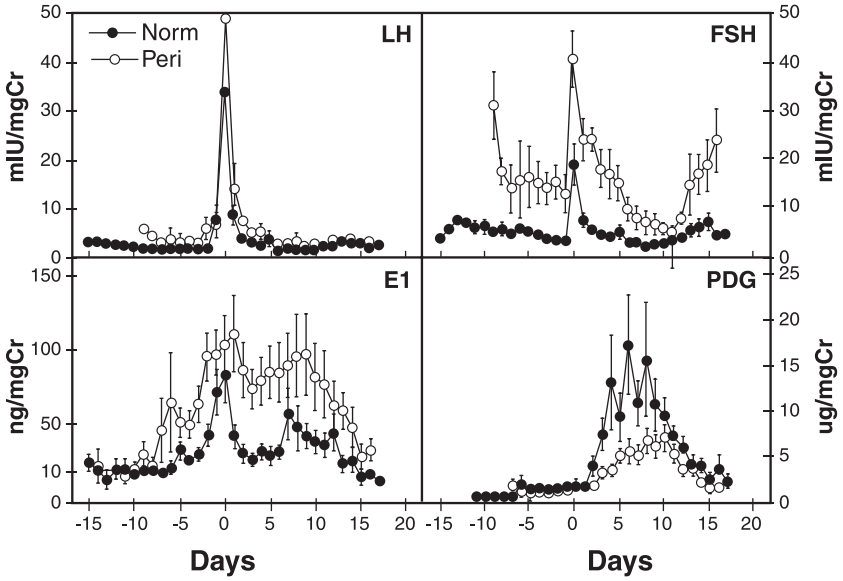

Figure 21.2 Daily urinary gonadotropin and sex steroid secretion patterns in perimenopausal women aged 43 compared to women between 19 and 38 years. Urinary FSH and estrone metabolites are significantly higher in perimenopausal women than in young controls. Data are standardized to day 0, the presumed day of ovulation and expressed as mean ± S.E. From Santoro *et al.,* 1996, Copyright 1996, The Endocrine Society.

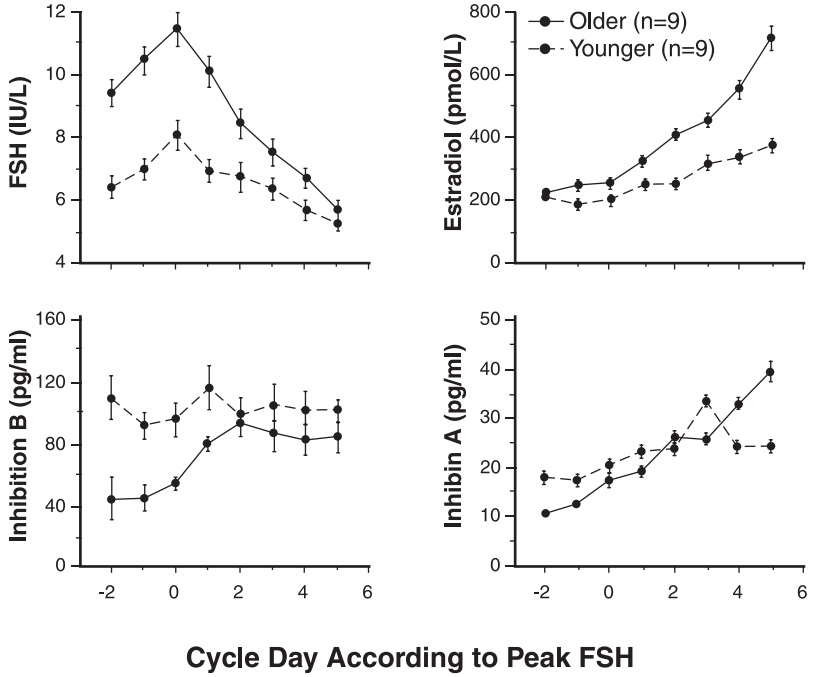

Figure 21.3 Plasma FSH (top left), estradiol (top right), inhibin B (bottom left), and inhibin A (bottom right) in younger (dashed lines) and older (solid lines) women. In older women, FSH concentrations are higher and inhibin B concentrations are lower than in young women. From Klein *et al.,* 1996, Copyright 1996, The Endocrine Society.

concentrations of FSH, unaccompanied by a rise in LH, becomes evident in both women (Klein *et al.*, 1996; Sherman & Korenman, 1975) and laboratory rats (DePaolo & Chappel, 1986) before any overt changes in cycle length. It is not caused by any loss in bioactivity of the FSH molecule, as there is no difference observed in the bioactive:immunoactive FSH ratio between women in their early twenties compared to those in their early forties (Klein *et al.*, 1996). This sentinel change is considered a predictor of impending reproductive decline and the marker that irregularity in cycle length will soon occur. As reproductive aging progresses, LH levels also increase, and changes in the pattern of LH secretion have been documented prior to the onset of perimenopause (Santoro *et al.*, 1996).

At later stages of the perimenopausal transition and postmenopausal period, multiple ovarian and pituitary hormones (Jaffe, 1999; Sherman *et al.*, 1976) exhibit changes in both concentrations and patterns of secretion. The detailed hormone profiles of Sherman and Korenman over the course of the perimenopausal period in individual women clearly establish that the pattern of ovarian steroid secretion that normally occurs over the course of a month is no longer predictable (see Figure 21.4). The highly

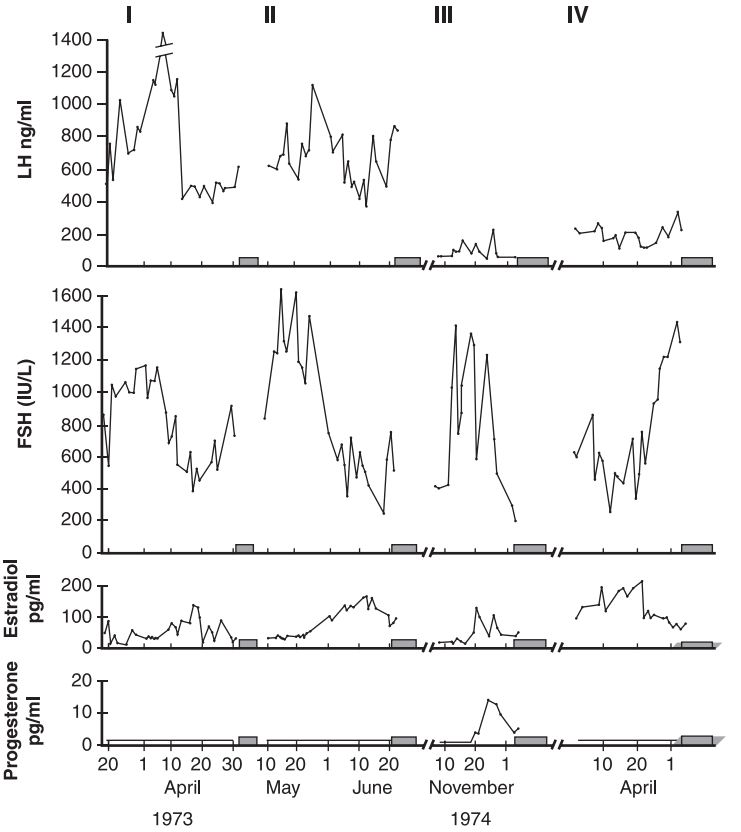

Figure 21.4 Daily concentration of serum luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, and progesterone during four cycles in one 49-year-old subject during the menopausal transition. Hormone levels are arrayed by calendar date, and the hatched areas indicate menstruation. During menopausal transition, hormone patterns show discordant regulation relative to the normal menstrual hormone relationship: FSH concentrations are elevated and not inhibited by high estrogen levels, whereas LH levels are in the normal or low range. From Sherman *et al.*, 1976, Copyright 1976, The Endocrine Society.

erratic concentrations, patterns, and relationships among the hormones raises the real possibility that all aspects of the reproductive axis are no longer coordinated in the normal feed-forward and feed-back manner. During postmenopause, estrogens decrease dramatically, and androgens, including testosterone and weaker androgens, decrease to a lesser magnitude (Zumoff *et al.*, 1995). Blood samples drawn from the ovarian vein compared to peripheral concentrations show that the majority of circulating estrogens are derived from peripheral conversion rather than ovarian synthesis (Judd & Korenman, 1982).

V. Role of the Central Nervous System in Female Reproductive Aging

A. Changes in Hypothalamic Signaling Contribute to Reproductive Aging

An alternative perspective that has gained attention is that the brain is a critical partner in reproductive aging: it is a pacemaker in the sequence of events leading to reproductive senescence. Several lines of evidence that will be discussed below support this contention. However, it should be emphasized that ultimately, exhaustion of ovarian follicles limits the reproductive life span. The elegant studies of Nelson, Felicio, and their colleagues emphasize the complexities of the partnership of the central nervous system and the ovary. These studies, performed in mice, led the authors to the conclusion that the hypothalamo-pituitary axis plays a role in the transition from regular to irregular estrous cycles during middle age, but the ovary is the primary factor in timing the actual cessation of ovulatory cycles. Parallel studies have not been performed in rats or nonhuman primate models.

It was once thought that the exhaustion of ovarian follicles was the most important factor leading to the transition to age-related permanent infertility. More recently, we realize that reproductive senescence is more complex and that there are probably multiple pacemakers involved in this process. What is clear from a wealth of data accumulated over many years is that an exquisite temporal order of signaling among the major components of the reproductive axis (brain, anterior pituitary, and ovary) is required for the occurrence of regular reproductive cycles. Of equal importance, the ultradian (intervals of minutes to hours) and diurnal (intervals of 24 hours) patterns and the amplitude of the hormonal excursions influence the regularity of cycles, the terminal stages of ovarian follicular growth and differentiation, and the occurrence of ovulation. Precise synchronization of the orchestrated ultradian, circadian, and infradian (intervals greater than 24 hours) events is the signature of the intricate communication required for successful reproductive cyclicity. Thus, the synthesis and secretion of gonadotropin releasing hormone (GnRH) from neurons within the hypothalamus are regulated by a repertoire of neurotransmitters. Which one(s) are primary and which are permissive are still not completely understood or universally accepted by investigators. The secretory pattern of GnRH determines the level of LH and FSH gene expression, their synthesis, their secretory patterns, the ratio of LH to FSH released, and the density of GnRH receptors in the pituitary gland. In turn, the patterns of gonadotropin secretion determine the success or failure of the final stages of follicular growth, development, and differentiation, and, hence, the pattern of steroid secretion. Most studies would suggest that the hormonal milieu does not influence the re-entry of dormant

primordial follicles into the growing pool and/or the initial stages of growth from primordial to primary or secondary follicles. However, controversy remains in this realm, and the final conclusions are not clear at the present time. The nature of steroid feedback—negative and positive—to the level of the anterior pituitary gland and hypothalamus is determined not only by the levels of steroid, but the duration of the elevation in steroid secretion and the ratio of estrogens/progestins and the temporal order of increases in these two steroids (see Knobil & Neill, 1994, for excellent chapter reviews of each of these topics).

Subtle changes in the temporal pattern and synchrony of neurochemical and neuroendocrine signals become detectable during middle age in both women (Matt *et al.*, 1998) and laboratory animal models (Wise *et al.*, 1997). They precede the cessation of reproductive cycles and may explain the accelerated loss of follicles that occurs during the perimenopausal period. The dynamics of specific neurotransmitters that regulate the secretion of GnRH changes with age (discussed below). However, the body of data suggests that it is more than any single neurotransmitter or neuropeptide that determines the role of the central nervous system in female reproductive aging. Multiple studies demonstrate that the temporal order and the pattern of multiple signals are altered during aging. These observations suggest that the dampening and desynchronization of the precisely orchestrated ultradian, circadian, and infradian neural signals lead to miscommunication between the brain and the pituitary-ovarian axis. In turn, an increasing desynchronization of neuroendocrine signals may contribute to the accelerated rate of follicular loss and the decreasing frequency of regular cycles that occurs during middle age. Direct measurement of neurotransmitter dynamics (e.g., release, the density of receptors, uptake of neurotransmitters at post-synaptic sites, and re-uptake at pre-synaptic sites) over a prolonged period remains methodologically impossible in humans. Perhaps in the future, *in vivo* imaging methods will allow us to monitor these changes in real time. However, until now, virtually all of the work that forms the foundation of this research has been performed in laboratory animal models. Studies in young, middle-aged, and old animals have revealed that age-related changes are progressive and more exaggerated in older rats that had completed the transition to acyclicity. These changes are subtle: investigators who measured indices of neural function at any one time of day in aging animals are unlikely to detect significant differences among age groups. However, together, disruption of the synchrony and coordination of multiple neural signals that regulate the precise timing of GnRH release may ultimately lead to important changes in the ability of rats to maintain regular estrous cycles.

B. Pituitary Hormone Secretion as a Surrogate of Brain Aging

GnRH is the primary hypothalamic hormone that regulates both LH and FSH secretion. In turn, its synthesis and secretion is regulated by a panoply of neurotransmitters and by estradiol negative feedback. Unfortunately, GnRH is not detectable in peripheral plasma because high concentrations exist only in the hypophysial portal blood, into which it is secreted from terminal boutons in the median eminence of the hypothalamus. Therefore, pulsatile patterns of secretion of LH have been used as a surrogate and are thought to reflect changes in the pattern of secretion of GnRH (Levine & Duffy, 1988). Changes in pulse amplitude

can result from changes at the hypothalamic and/or pituitary level, whereas changes in the inter-pulse interval and duration of pulses are thought to reflect more purely changes in the hypothalamic pulse generator and the accuracy with which it generates discrete, robust signals.

Changes in the patterns of pulsatile LH secretion have been detected in both perimenopausal women and middle-aged rats. In women, reports of changes in the pattern of pulsatility are contradictory. Matt and colleagues (1998) reported that in middle-aged women whose menstrual cycles remain the normal length, the frequency of LH pulses decreases and the width of the peak increases prior to any change in plasma estradiol. However, when the menstrual cycle length shortens, Reame and colleagues (1996) reported that LH pulse frequency was higher in older women. Similar changes have been reported in rodent models. Scarbrough and Wise (1990) monitored pulsatile LH release in ovariectomized young and middle-aged rats and found that the inter-pulse interval and average duration of individual pulses increased (see Figure 21.5). As in studies performed in women, these results from studies performed in rats strongly suggest that subtle changes in the integrity of the GnRH pulse generator occur early, prior to the transition from regular to irregular cycles, and may be a component of the cascade of events that contribute to reproductive aging.

C. GnRH Secretion During Aging

As we focus our attention on potential changes that occur at the level of the central nervous system, it is important to emphasize that virtually all of these studies have been performed in laboratory rodents. Very recently, Gore and colleagues (2004) have published work on changes in pulsatile GnRH secretion in the aging female Rhesus monkey. It is possible that not all of the factors that regulate GnRH secretion in rodents and nonhuman primates are identical to those that regulate secretion in humans. However, these species have been excellent experimental models and have provided important insights into the mechanisms and factors that regulate development of the reproductive system, puberty, and maintenance of regular cycles in the adult. We anticipate that the information gained from these species can be applied to humans and will provide an understanding that can be generalized to human reproductive aging.

Even in rodent models, it has been problematic to monitor GnRH activity and secretion. Only between 1,000 to 2,000 GnRH neurons exist in the brain, and they are widely and diffusely distributed through the septo-preoptico-infundibular pathway of rodents and the medial basal hypothalamus of humans (Silverman, 1994). Furthermore, because GnRH receptors are expressed in diverse regions of the brain that do not appear to be in anatomical locations that could influence pituitary gonadotropin secretion, it is thought that GnRH may have multiple functions, not all of which are directly related to gonadotropin secretion. We do not know whether anatomically distinct subpopulations of GnRH neurons are specifically dedicated to regulating LH and FSH, although recent data (Petersen *et al.*, 1993; Rance & Uswandi, 1996) suggest that they may exist. For all of these reasons, it has been extremely difficult to correlate GnRH activity patterns over time in individual animals under controlled experimental conditions, although a few investigators have successfully achieved this technically challenging feat in rats (e.g., Levine & Duffy, 1988; Levine & Ramirez, 1982; Rubin & Bridges, 1989) and monkeys (Terasawa, 1995). Other methods, including quantitation of

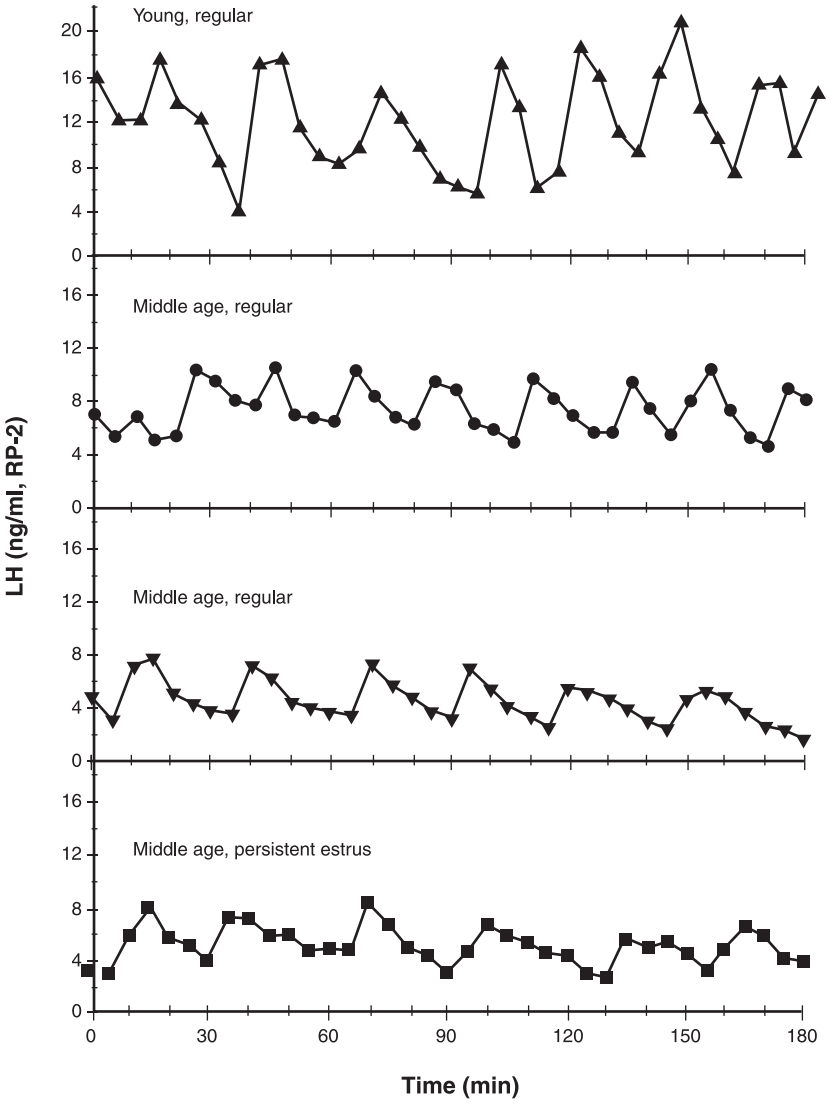

Figure 21.5 LH concentrations are shown from a representative young regularly cycling and middle-aged rats at various stages of reproductive senescence (regularly cycling, irregularly cycling, and acyclic persistent estrous). All rats were ovariectomized for four weeks prior to blood collection. Blood samples were collected at five-minute intervals for a three-hour period. From Scarbrough & Wise, 1990, Copyright 190; The Endocrine Society.

mRNA levels to assess gene expression and use of dual label immunocytochemistry to identify fos expression in GnRH neurons, an index of activation, have allowed us to monitor the activity of GnRH neurons in aging animals. Rubin and Bridges (1989) reported alterations in GnRH release from the mediobasal hypothalamus of steroid-primed middle-aged rats, as monitored by push-pull cannula methods. These functional changes become apparent prior to any detectable change in the number of immunoreactive GnRH neurons (Lloyd *et al.*, 1994), in morphology or distribution of GnRH neurons of aging male

rats (Witkin, 1987), or any age-related differences in the distribution of GnRH-immunoreactive forms expressed in GnRH neurons (Hoffman & Finch, 1986). Thus, functional changes in GnRH neurons appear to precede changes in the ability to maintain regular estrous cyclicity and appear to be a more sensitive measure of the status of GnRH neuronal activity than morphological criteria or alterations in the absolute concentrations of GnRH.

Equivalent studies are impossible in humans. To our knowledge, only two investigations that assessed GnRH have been performed in human females. Both monitored GnRH in postmenopausal women; no studies have assessed GnRH neuronal changes prior to or during the perimenopausal transition. The existing data are contradictory: Parker and Porter (1984) reported that radioimmunoassayable GnRH concentrations in the mediobasal hypothalamus were lower in postmenopausal women than in young. More recently, Rance and Uswandi (1996) found that GnRH mRNA levels in the tuberoinfundibular region were elevated in postmenopausal women. A possible interpretation of these seemingly contradictory findings is that, in the presence of low estrogen characteristic of the postmenopausal state, transcription of the GnRH gene increases, and release of the peptide is elevated to an even greater extent, such that steady-state mRNA levels are elevated but the stored pool of GnRH in the mediobasal hypothalamus is lower than in young women. Obviously much more needs to be done before we can clearly interpret these data or draw conclusions as to the factors that lead to such changes.

Observations that rhythmicity is altered have led investigators to question whether deterioration of a master pacemaker may explain the desynchronization of multiple neuroendocrine rhythms. The suprachiasmatic nucleus (SCN) of the hypothalamus is considered the master circadian pacemaker, or biological clock, in mammals (Moore-Ede *et al.*, 1982; Turek & Van Cauter, 1994). These bilateral nuclei, which are located at the base of the brain dorsal to the optic chiasm, exhibit endogenous circadian rhythmicity: they continue to exhibit circadian electrophysiological activity and neuropeptide secretion patterns even when removed and maintained *in vitro* (Turek, 1985). Neurons from the SCN communicate extensively with each other, send efferents to many regions of the brain, and drive the timing of multiple outputs so that almost all physiological functions show a pervasive daily rhythm. For the female reproductive system to maintain regular cycles, the circadian system must be intact. This is most evident in laboratory animals that are maintained in controlled laboratory conditions (Everett & Sawyer, 1950; Legan & Karsch, 1975). However, even in humans where activity and light-dark and sleep-wake cycles are not rigorously controlled, reproductive functions exhibit a diurnal rhythmicity (Casper *et al.*, 1988; Czeisler *et al.*, 1990; Khoury *et al.*, 1987; Testart *et al.*, 1982). The biochemical mechanism by which time-of-day information is transmitted from the SCN to other regions of the brain is through several key neuropeptides. They are an integral part of the inputs or outputs of the clock. The SCN sends projections directly to GnRH neurons (Hoorneman & Buijs, 1982; van der Beek *et al.*, 1993) and may communicate temporal information to the reproductive axis. Thus, deterioration in this neural pacemaker or the coupling to its outputs may initiate the gradual disintegration of the temporal organization of neurotransmitter rhythms that are critical for stable, precise, and regular cyclic GnRH secretion. In turn, this deterioration and desynchronization of multiple

neuropeptides of the clock may initiate a cascade that leads to the transition to irregular cycles and ultimately contributes to acyclicity. Several lines of evidence suggest that aging results in a decline of this critical master pacemaker and that this leads to a desynchronization of multiple physiological rhythms, including ones that are required for cyclic gonadotropin secretion and follicular development and differentiation.

Numerous reports that multiple circadian rhythms are compromised in aging organisms support the concept that the clock itself or its coupling to an array of outputs may deteriorate with age: the period of rhythms decrease, the phase of many outputs of the clock advances, and the amplitude of multiple rhythms is attenuated with age (see Figure 21.6) (for review, see Brock, 1991; Richardson, 1990). In addition, temporal desynchronization of two or more rhythms, fragmentation of circadian rhythms, and altered responsiveness to stimuli that induce phase shifts are a common occurrence in older organisms. Thus, declining reproductive function may be only one of many physiological endpoints to suffer from the fragmentation of temporal organization of physiological functions.

There appears to be a difference between aging of the reproductive system in humans and rats in the secretory patterns of LH. In women, most reports suggest that the average plasma LH levels do not rise until later during the menopausal transition, despite alterations in FSH secretion. Korenman and colleagues (1978) monitored LH levels in individual women over several months during the perimenopausal transition. These studies show that LH levels are highly variable during this period and cannot be consistently explained by the changes in estradiol negative or positive feedback. No changes have been observed in the average concentrations of LH secreted during the preovulatory surge (Klein *et al.*, 1996; Korenman *et al.*, 1978). In contrast, in rats, the preovulatory LH surge is both delayed and attenuated in middle-aged rats prior to overt changes in the length or regularity of the LH surge (Cooper *et al.*, 1980; Nass *et al.*, 1984; Wise, 1982a). Intriguingly, Nass and colleagues (1984) found that regularly cycling rats that were destined to become irregular cyclers exhibited delayed and attenuated LH release compared to those that would continue to cycle for at least the following six months.

D. Multiple Neurotransmitters that Regulate GnRH Change with Age

Changes in the pattern of GnRH expression and secretion may result from changes in one or more of the repertoire of neurotransmitters and neuropeptides that modulate neuronal activity. Investigators have examined monoamine activity, neurotransmitter receptor densities, and the gene expression of some of the neuropeptide neuromodulators of GnRH. It appears that during middle age, the diurnal rhythmicity in the activity of many neurotransmitters, the density of their receptors, and/or the level of gene expression is dampened or undetectable in hypothalamic regions involved in regulating the pattern of GnRH neuronal activity. Age-related changes have been detectable by the time animals were middle-aged, as they were entering the transition to irregular cycles. For example, changes in the pattern of proopiomelanocortin (POMC) gene expression can be used as an example of the many neurochemical events that exhibit changes in rhythmicity. In young rats, POMC gene expression exhibited a diurnal rhythm (see Figure 21.7). This rhythm was undetectable when rats were middle-aged or older. Similar age-related changes have been reported in many of the neuromodulators of GnRH release. Thus, it would appear that, during middle age,

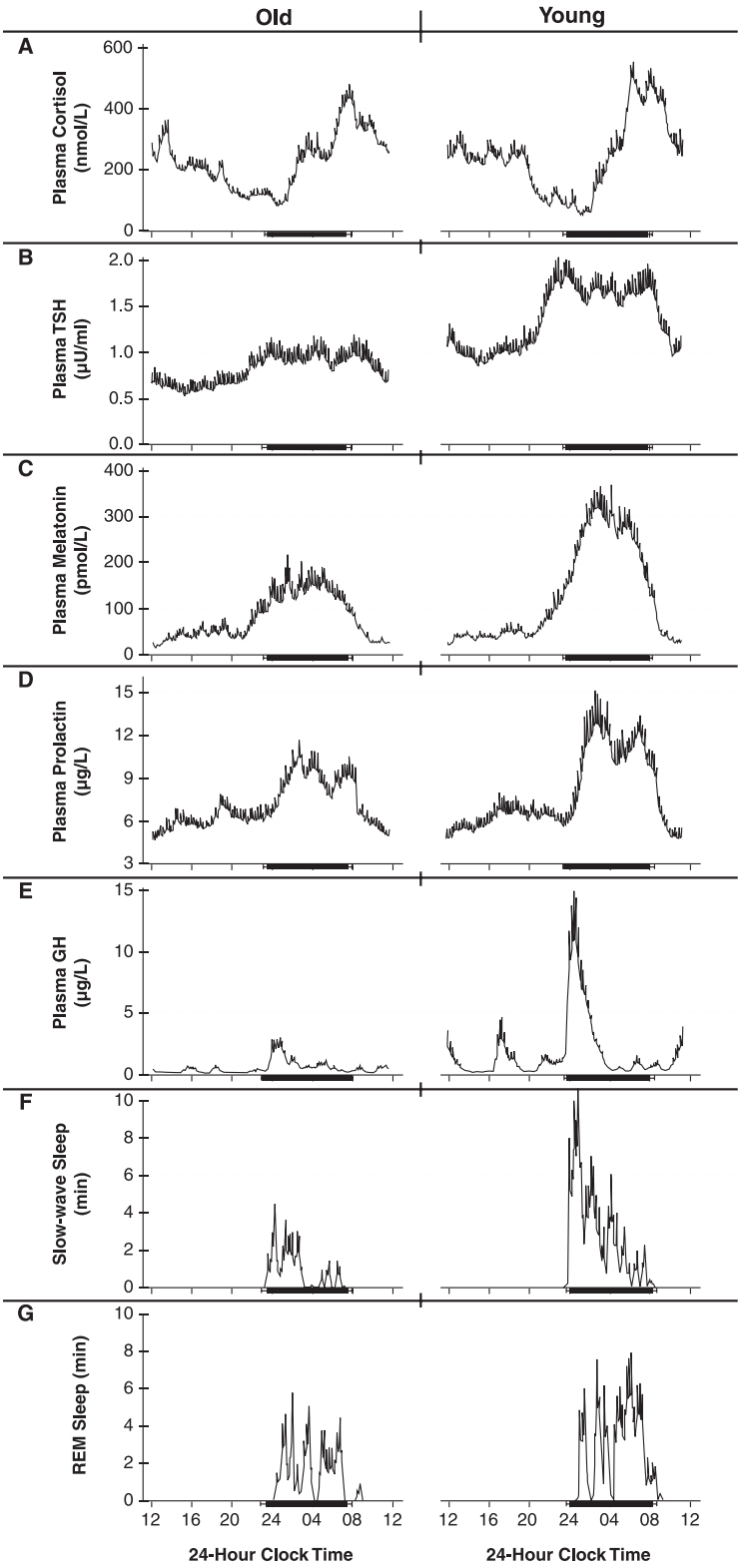

Figure 21.6 Twenty-four-hour profiles of plasma cortisol (A), thyroid-stimulating hormone TSH (B), melatonin (C), prolactin (D), and growth hormone (GH) (E) levels and distribution of slow wave (SW) (F), and rapid-eye movement (REM) (G) stages in old and young subjects. Distribution of sleep stages is expressed in minutes in each 15-minute interval between blood samplings spent in SW or REM stage. Mean ± S.E. black bars correspond to mean sleep period. From van Coevorden *et al.*, 1991, Copyright 1991, The American Physiological Society.

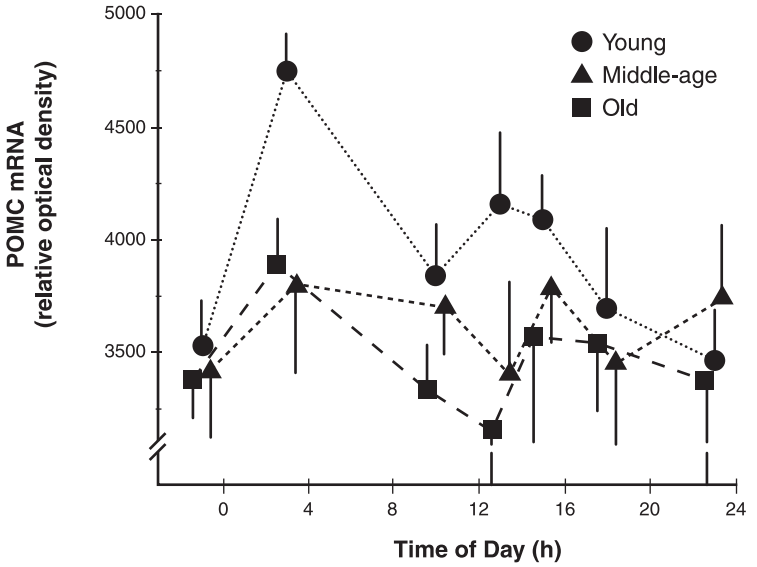

Figure 21.7 Proopiomelanocortin (POMC) mRNA concentrations in the arcuate nucleus of young (circles), middle-aged (triangles), and old (squares) rats. In young rats, POMC mRNA levels exhibit diurnal rhythmicity. This rhythm disappears by the time animals become middle-aged (mean ± S.E.). From Weiland *et al.*, 1992, Copyright 1992, The Endocrine Society.

the precise, synchronized, and interactive patterns of hypothalamic neurotransmitter and neuropeptide activity, which are critical to maintain a specific pattern of GnRH secretion, become less ordered. Similar changes may occur in humans and may be manifested by the occurrence of hot flushes, a hallmark of deterioration of the hypothalamic thermoregulatory centers. Some researchers propose that this deterioration in communication among the neurotransmitters that regulate GnRH secretion causes the initial changes in patterns of gonadotropin secretion and that these changes herald the imminent transition to the perimenopausal state.

VI. Conclusion

In summary, considerable evidence has accumulated that both the ovary and the brain exhibit changes during aging. The most recent studies show that multiple events at different levels of the reproductive axis lead to reproductive decline. The roles of the brain and ovary may be different: the brain may be more involved in the initial deterioration in reproductive cycle regularity, whereas the ovarian follicular reserve may be the ultimate driver of the cessation in cyclicity. Our goals for the future are to better understand the repertoire of factors that interact to maintain regular reproductive cyclicity and how this dynamic balance changes with age. It will be important to determine which alterations are primary and cause decline in reproduction and which are secondary correlates of the primary changes.

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