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The role of the brain in female reproductive aging

Jodi L. Downs[1,*] and **Phyllis M. Wise**[1,2,3]

1*Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195, USA*

2*Department of Obstetrics and Gynecology, University of Washington, Seattle, WA 98195, USA*

3*Department of Biology, University of Washington, Seattle, WA 98195, USA*

Abstract

In middle-aged women, follicular depletion is a critical factor mediating the menopausal transition; however, all levels of the hypothalamic-pituitary-gonadal (HPG) axis contribute to the age-related decline in reproductive function. To help elucidate the complex interactions between the ovary and brain during middle-age that lead to the onset of the menopause, we utilize animal models which share striking similarities in reproductive physiology. Our results show that during middle-age, prior to any overt irregularities in estrous cyclicity, the ability of 17β-estradiol (E_2) to modulate the cascade of neurochemical events required for preovulatory gonadotropin-releasing hormone (GnRH) release and a luteinizing hormone (LH) surge is diminished. Middle-aged female rats experience a delay in and an attenuation of LH release in response to E_2. Additionally, although we do not observe a decrease in GnRH neuron number until a very advanced age, E_2-mediated GnRH neuronal activation declines during the earliest stages of age-related reproductive decline. Numerous hypothalamic neuropeptides and neurochemical stimulatory inputs (i.e., glutamate, norepinephrine (NE), and vasoactive intestinal peptide (VIP) that drive the E_2-mediated GnRH/LH surge appear to dampen with age or lack the precise temporal coordination required for a specific pattern of GnRH secretion, while inhibitory signals such as gamma aminobutyric acid (GABA) and opioid peptides remain unchanged or elevated during the afternoon of proestrus. These changes, occurring at the level of the hypothalamus, lead to irregular estrous cycles and, ultimately, the cessation of reproductive function. Taken together, our studies indicate that the hypothalamus is an important contributor to age-related female reproductive decline.

Keywords

aging; brain; estradiol; female; neuroendocrine; reproduction

1. Introduction

Although the mean life span of humans continues to increase, the mean age at which women begin the perimenopausal transition has remained constant at 45.5-47.5 years, a process that lasts about 4 years (Burger et al., 2002; Hidayet et al., 1999; McKinlay et al., 1992; Treloar, 1981). Therefore, most women can expect to spend over one third of their lives in the

*To whom correspondence should be addressed: Jodi L. Downs, Ph.D., Department of Physiology and Biophysics, University of Washington, 1959 NE Pacific Street, Box 356460, Seattle, WA 98195, Telephone: (206) 543-9970, FAX: (206) 534-5480, E-mail: E-mail: jldowns@u.washington.edu.

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postmenopausal state where the associated chronic decrease in E_2 will have far-reaching health implications on bone (Ebeling et al., 1996; Seifert-Klauss et al., 2002), neurodegeneration and stroke (Suzuki et al., 2006; Suzuki et al., 2007; Wise et al., 2005), cognition (Halbreich et al., 1995; Lacreuse, 2006; Paganini-Hill and Henderson, 1994; Rapp et al., 2003; Roberts et al., 1997; Shaywitz et al., 1999; Voytko and Tinkler, 2004), cardiovascular disease (Do et al., 2000; Hall et al., 2002; Luoto et al., 2000; Matthews et al., 2001), and immune function (Keller et al., 2001; Pfeilschifter et al., 2002; Porter et al., 2001). For these reasons, factors underlying the timing of the onset of the menopause and the repercussions of attenuated circulating E_2 on women's health are of considerable interest. We utilize animal models to gain insight about the complex interactions between the ovary and the brain leading up to the onset of the menopause and the hypoestrogenic state. During the past five years, we have come to appreciate that during middle-age, the changes that occur in reproductive cyclicity and hormone patterns among women, nonhuman primates, and rodents are strikingly similar (Bellino and Wise, 2003; Downs and Urbanski, 2006; Wise et al., 1999; Wise et al., 2002; Wu et al., 2005). In this review we include a discussion of the differences and similarities of rodent and nonhuman primate model systems for human menopause onset. This review focuses primarily on our work; however, we cite numerous studies from our colleagues that have greatly benefited our understanding of neuroendocrine factors contributing to female reproductive senescence.

2. Aging of the female hypothalamic-pituitary-ovarian axis

Age-related female reproductive decline involves deficits at all levels of the HPG axis (Peng and Huang, 1972; Rubin, 2000; Wise et al., 1999; Wise et al., 2002). In women, depletion of the postmitotic pool of ovarian follicles and the associated decline in circulating E_2 concentrations are traditionally recognized as being the ultimate markers of menopause (vom Saal, 1994). In addition to the ovary, the pituitary is likely to contribute to reproductive decline since the LH response to a pharmacological doses of GnRH administered in the early follicular phase decreases in older compared with younger cycling women (Fujimoto et al., 1993; Weiss et al., 2004). However, accumulating evidence indicates that during the earliest stages of reproductive decline, prior to any changes in ovarian cyclicity and circulating E_2, changes at the level of the brain play an important role in the initiation of reproductive senescence (Gore et al., 2004; Lapolt and Lu, 2001; Lu et al., 1994; Rubin, 2000; Wise, 1993; Wise et al., 1997; Wise et al., 2002; Yin and Gore, 2006).

One of the earliest markers of reproductive decline is a dampened and delayed GnRH-driven proestrous LH surge (Lu, 1983;Nass et al., 1984;van der Schoot, 1976;Wise, 1982). Prior to any observable changes in estrous cycle length or regularity, we observed a consistent delay in the onset of the LH surge and an attenuation in peak LH concentrations (Fig. 1). These age-related changes in LH release likely reflect alterations at the level of the hypothalamus rather than the pituitary because they occur at an age prior to any change in pituitary responsiveness to GnRH. However, since we have never observed a change in GnRH neuron number during middle-age (Krajnak et al., 2001;Lloyd et al., 1994), an age-related change in GnRH neuronal activity is more likely to contribute to the alterations in LH release that we observe. To determine if a change in GnRH neuronal function during middle-age occurs, and if such a change could result in a dampening and delay of the LH surge, we quantified the expression of the immediate early gene, cFos, as an indicator of GnRH neuronal activity. We used this immunocytochemical technique, which has been used to identify increased neuronal activity in individual neurons (Hoffman et al., 1993) because GnRH is not detectable in peripheral blood, GnRH neurons are few in number, and their populations are diffuse. In Fig. 2, we show that GnRH neuronal activation in middle-aged, regularly cycling female rats was significantly lower compared to their young counterparts during the time of the proestrous LH surge (Lloyd et al., 1994). Others have confirmed and extended our results by using three-dimensional reconstructions of GnRH populations in young and middle-aged rats showing similar age-

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related declines in GnRH neuronal activation either during the proestrous or E_2-induced LH surge (Rubin et al., 1994;Rubin et al., 1995). These findings strongly indicate that afferent inputs to GnRH neurons change during the earliest stages of reproductive aging.

3. Neuronal factors involved in the onset of reproductive senescence

The driving force behind GnRH/LH output relies upon the complex balance between, and timing of, stimulatory and inhibitory inputs to GnRH neurons. By middle-age the orchestration of this balance has deteriorated, which is manifested in reduced GnRH release, leading to a reduction in magnitude and delay in the LH surge (Wise, 1982; Wise et al., 2002). Much attention has been paid to the role of declining stimulating factors that may contribute to the observed age-effects on the GnRH/LH surge. For example, excitatory input from the amino acid, glutamate, is a critical stimulatory factor that directly influences GnRH neuronal activity through the *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors (Gore, 2004). However, during aging glutamatergic input to brain regions containing GnRH neurons appear to decrease (Brann et al., 2005; Neal-Perry et al., 2005). Additionally, glutamatergic mediated GnRH release (Zuo et al., 1996) and glutamate receptor subunits, expressed on GnRH neurons, appear to decline with age (Gore et al., 2000; Smith and Jennes, 2001), possibly contributing to the delay and decline in the GnRH/LH surge.

Another key stimulator of GnRH release is norepinepherine (NE), which acts through the α_1-adrenergic receptor and is important in the induction of the GnRH/LH surge (Barraclough and Wise, 1982; Barraclough et al., 1984). We observed that NE exhibits a diurnal rhythm in which the activity turnover rate is increased just prior to and during the LH surge in young but not middle-aged rats (Rance et al., 1981; Wise, 1982; Wise, 1984). Moreover, the regions where we observed an age-related dampening of the diurnal variation in NE turnover occurred in key anterior hypothalamic nuclei that regulate the timing of the LH surge (Wise, 1984). Additionally, with age, the density of the α_1-adrenergic receptors lacks the diurnal variation observed in several hypothalamic nuclei of young rats (Weiland et al., 1989; Weiland and Wise, 1990). The loss of rhythmic monoamine turnover (Wise, 1984) and adrenergic receptor density (Weiland et al., 1989; Weiland and Wise, 1990), in conjunction with a reduced sensitivity of NE neurons to E_2 (which may contribute to lower catecholamine release during the LH surge in middle-aged rats) (Temel et al., 2002), strongly suggest that the subtle control of NE is compromised during the earliest stages of reproductive decline.

However, in order for stimulating factors such as NE to have their full effect on GnRH neurons to initiate the GnRH/LH surge, a withdrawal of inhibitory factors on GnRH release appears to be necessary (Kalra and Kalra, 1984). Additionally, pharmacological blockade of inhibitory tone on GnRH during early proestrus results in an advance in the LH surge. Therefore, investigating age-effects on inhibitory factors contributing to restraining GnRH release seem equally important as age-effects on GnRH stimulatory factors.

Gamma-aminobutyric acid (GABA) and opioid peptides act as inhibitory neurotransmitters and as important modulators of the timing and amplitude of the GnRH/LH surge on proestrus (Cashion et al., 2004; Kalra and Kalra, 1984). During the estrous cycle, GABA and opioid peptides act to restrain GnRH secretion, but as proestrus nears, their activity lessens, allowing for stimulating neuromodulators to maximally influence GnRH neurons. We (Cashion et al., 2004), and others (Grove-Strawser et al., 2007) observe a loss of rhythmic GAD_{67} gene expression, the rate-limiting enzyme for GABA synthesis, with age. Studies suggest that multiple neurotransmitters that modulate GnRH synthesis and secretion may account for changes in GnRH dynamics. The long list of neurotransmitters and neuropeptides that alter the release of GnRH continues to grow while the hierarchy of neuromodulators remains unclear.

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The sensitivity to E_2 positive feedback declines with age in the female rat, resulting in an attenuated LH surge that is delayed (Wise, 1984; Wise et al., 1996). Similar findings in perimenopausal women indicate that the ability of exogenous E_2 to induce a surge is diminished (van Look et al., 1977). It is likely that the important hypothalamic nodal point for the proper convergence of circadian signals from the SCN and E_2 stimulation is the anteroventral periventricular (AVPV) nucleus and that a decline in the responsiveness of GnRH/LH to E_2 with age may be at least partially attributable to age-related changes within the AVPV. We know that the AVPV expresses estrogen receptors (Petersen et al., 2003) and if lesioned, GnRH/LH surges are abolished (Wiegand and Terasawa, 1982). Moreover, we have shown that E_2-induced activation of the medial AVPV and GnRH neurons decline in parallel in middle-aged rats (Le et al., 2001). This suggests that an age-related deficit in E_2 responsiveness at the level of the medial AVPV may facilitate, in part, the age-related delay in and attenuation of the E_2-induced GnRH/LH surge.

4. Aging of circadian signals contribute to reproductive decline

Timing is a critical factor in initiating the preovulatory GnRH/LH surge (Chappell, 2005; de la Iglesia and Schwartz, 2006). A series of seminal studies by John Everett and Charles Sawyer first established that two requirements were necessary to induce a preovulatory LH surge in the female rat: 1) a neurogenic signal during a “critical period”; and 2) a rise in circulating E_2 (Everett et al., 1949; Everett and Sawyer, 1950; Everett and Sawyer, 1953; Sawyer et al., 1949). These studies indicated that the neuronal signal(s) leading to the LH surge needed to occur within a precisely timed window, and if they did not, the surge was delayed 24 hours, implicating a circadian neuronal signal as a prerequisite. An extension of the 4-day estrous cycle of the rat is intrinsically unstable due to uncoupling of the temporally coordinated rise in E_2, the light/dark cycle, circadian signal and the neurogenic critical period (Schwartz, 1969). The mechanisms underlying how these two necessities integrate within the hypothalamus to elicit an appropriately timed GnRH/LH surge and how this mechanism changes with age remains an area of active research. Subsequent evidence indicated that the circadian pacemaker, located in the suprachiasmatic nuclei (SCN), regulates the timing of the preovulatory and steroid-induced LH surge. Researchers found:

1. direct neuronal pathways from the SCN to GnRH neuronal populations exist (Krajnak et al., 2001; van der Beek et al., 1997), and E_2-receptive neurons in the AVPV are synaptic targets of the SCN (Watson et al., 1995).
2. rodents that are ovariectomized and treated with E_2 have daily afternoon surges of LH that occur at a specific time relative to the light/dark cycle, indicating that the circadian signal is produced daily, but requires high E_2 levels to be expressed (Legan and Karsch, 1975; Legan et al., 1975).
3. lesions of the SCN abolish estrous cycles in gonadally intact females (Gray et al., 1978; Stetson and Watson-Whitmyre, 1976; Terasawa et al., 1980) and eliminate daily afternoon surges of LH in E_2-treated ovariectomized females (Kawakami et al., 1980). Thus, we can conclude that the rhythm that regulates the LH surge and estrous cycle is generated endogenously by a circadian pacemaker located in the SCN and that the AVPV is a likely intermediary.

Coupling of a circadian neuronal signal(s) from the mammalian biological clock, or SCN, with GnRH neuromodulators induces the E_2-driven GnRH/LH surge (Chappell, 2005). Several lines of evidence indicate that the rhythmic expression of the neuropeptide, vasoactive intestinal polypeptide (VIP), primarily from the ventrolateral SCN, acts to directly convey time-of-day information to GnRH neurons in the female rat (van der Beek, 1996). Data show that: 1) GnRH neurons receive direct innervations from VIP afferents (van der Beek et al., 1993), 2) approximately 40% of GnRH neurons express the VIP_2 receptor (Smith et al., 2000), and 3)

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GnRH neurons that are innervated by VIP fibers are preferentially activated during an E_2-induced GnRH/LH surge (van der Beek et al., 1994). Furthermore, an uncoupling of the rhythmic VIP signal to GnRH neurons occurs during middle-age, which appears to partially account for the delay and attenuation of the LH surge in E_2-treated female rats. This line of reasoning is supported by our observations that a dampening of the VIP rhythm occurs with age (Fig. 3); conversely, the arginine vasopressin (AVP) rhythm is preserved during aging (Krajnak et al., 1998). These differential age-dependent effects suggest that the integrity of the SCN does not deteriorate in a uniform manner. We observed a clear attenuation of the VIP mRNA rhythm, while the number of AVP cells, predominantly located in the dorsomedial aspect of the SCN, and the amount of AVP mRNA per cell were unaffected with age. In view of the fact that the rhythm of VIP expression is lost with advancing age, we sought to determine if a decrease in VIP and suppression of its rhythm would lead to a decrease/delay in the E_2-mediated LH surge. We found that the effects of age on the LH surge could be mimicked in young, ovariectomized, E_2-treated rats when the VIP signal was suppressed by direct infusion of VIP antisense oligonucleotides into the SCN (Fig. 4). Rats treated with antisense oligonucleotides to VIP displayed a decrease and delay in their E_2-mediated LH surge (Harney et al., 1996). Our observations were later supported when the immunoneutralization of VIP by the central administration of antiserum to VIP reduced and delayed the E_2-induced LH surge in rats (van der Beek et al., 1999).

We next sought to determine if advancing age could alter the innervation of VIP fibers onto GnRH neurons and the sensitivity of GnRH neurons to VIP (Fig. 5). Using immunohistochemical techniques, we found that the number of GnRH neurons that are likely to receive direct VIP input due to their close apposition does not decrease with age. However, the number of activated GnRH neurons determined by Fos expression closely apposed by VIP fibers was significantly decreased during the peak of an E_2-induced LH surge (Krajnak et al., 2001). Taken together, these results provide further evidence for direct VIP innervation from the SCN to GnRH neurons and that the age-related attenuation and delay in the LH surge is not due to a decline in VIP input to GnRH, but may rather be due to a decreased sensitivity of GnRH neurons to VIP.

5. Experimental models to study the role of the brain in female reproductive aging

Rodents

The majority of information on which we base our conclusions regarding the brain's role in the onset of reproductive senescence originates from studies performed in rodents. Some controversy exists as to whether the rodent provides a suitable model for studying reproductive aging in women. Questions surrounding the appropriateness of the rodent model to provide insights into the onset of menopause in women stem from two primary observations. First, the negative feedback effects of E_2 on gonadotropins in the aging rat are different than those observed in women. As the levels of ovarian-derived E_2 drop precipitously in the postmenopausal woman, circulating LH and follicle-stimulating hormone (FSH) become unrestrained and are both markedly elevated (Yen, 1999). Conversely, circulating LH concentrations remain relatively normal in aged, acyclic, repeatedly pseudopregnant rats (Lu, 1983) despite an age-related decline in E_2. These data suggest that a decline or disconnect in hypothalamic drive to GnRH neurons during reproductive aging may be fundamentally critical in rats but less so in women. Second, the age-related dynamics of the depletion of the ovarian follicular reserve may be different between rodents and women. As women approach middle-age, the depletion of the follicular pool appears to accelerate, so by the end of a woman's reproductive life she is left with virtually no ovarian follicles (Richardson et al., 1987). In

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contrast, although an equivalent study in rats has not been performed, some follicles remain in old acyclic rats (Lu et al., 1979).

Despite key differences in the roles of the brain, pituitary and ovary in the postreproductive states of acyclic female rats and postmenopausal women, there are remarkable parallels between middle-aged female rats and middle-aged pre- and peri-menopausal women (Rubin, 2000; Wise et al., 2002). First, one of the earliest hallmarks of impending reproductive decline in both women and female rats is a rise in FSH, especially near the time of ovulation (Reyes et al., 1977; DePaolo and Chappel, 1986; DePaolo, 1987; Klein et al., 1996). Second, the interpulse interval and pulse duration of LH release increases similarly in older regularly cycling women (Matt et al., 1998) and middle-aged female rats (Scarbrough and Wise, 1990). Third, reproductive cycle length becomes highly variable in women and middle-aged female rats as they progress towards reproductive decline (Fitzgerald et al., 1994; Sherman et al., 1976). Fourth, pre- and peri-menopausal women have normal, or even elevated, circulating E_2 concentrations (Klein et al., 1996; Santoro et al., 1996) as do middle-aged rats as they begin the transition to irregular estrous cyclicity (Butcher and Page, 1981; Lu, 1983). Additionally, the capacity of E_2 to induce GnRH/LH surges is diminished in middle-aged rats and perimenopausal women (van Look et al., 1977; Weiss et al., 2004; Wise, 1984). And finally, aging alters the timing and the amplitude of the LH surge such that the onset and peak are delayed and the amplitude is diminished (Wise, 1982; Wise, 1984). These key pieces of evidence emphasize that changes at the level of the brain may drive the earliest stages of the transition to reproductive decline in both women and female rats, including the accelerated follicular atresia that occurs in middle-aged women (Faddy et al., 1983; Richardson et al., 1987).

Nonhuman Primates

Like women, female rhesus monkeys experience regular 28-day menstrual cycles and sloughing of the endometrial lining. With advanced age, female rhesus monkeys undergo the transitions through menopause, culminating in the loss of menstrual cycles and the associated decline in E_2 (Downs and Urbanski, 2006). Moreover, aged female rhesus monkeys experience a marked elevation in circulating FSH concentrations prior to any overt changes in menstrual cyclicity or circulating E_2 concentrations (Downs and Urbanski, 2006), which acts as a hallmark of impending reproductive decline in women (Reyes et al., 1977). Despite these similarities that make the rhesus monkey a valuable model of menopause in women, nonhuman primate research can be prohibitive because of cost and the time required to investigate aging systems in such a long-lived species. Although these caveats have limited the extent of nonhuman primate studies regarding the role of the brain in the onset of reproductive aging, some important contributions have been made utilizing the monkey as a model system. Pulsatile GnRH release increases during the menopausal transition in the nonhuman primate (Gore et al., 2004), and parallel changes in pulsatile LH release have been detected (Woller et al., 2002). Whether these age-related changes precede age-related ovarian hormonal changes is yet to be determined. Taken together, some, but not all, parameters are shared between rats, nonhuman primates and human females during the earliest stages of reproductive decline prior to the onset of any detectable changes in reproductive cyclicity or decline in E_2, of which the robust monotropic rise in FSH (Fig. 6) is one of the earliest detectable.

5. Summary

The complex and redundant mechanisms governing female reproduction emphasize that no single piece of the HPG axis can completely explain the changes that occur to mediate age-related reproductive decline. Evidence indicate female reproductive aging involves all levels of the HPG axis from studies in several species, including rats, monkeys, and women. The

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balance of stimulatory and inhibitory GnRH neuromodulators becomes temporally disorganized and deteriorates with advancing age. We propose that this imbalance leads to an insensitivity to E_2 and an uncoupling from critical circadian signals and is therefore what causes the attenuation and delay of the E_2-induced GnRH/LH surge leading to reproductive senescence. Future studies that exploit the advantages of different animal models in cooperation with clinical investigations will undoubtedly shed further light on the key factors that orchestrate the onset of menopause.

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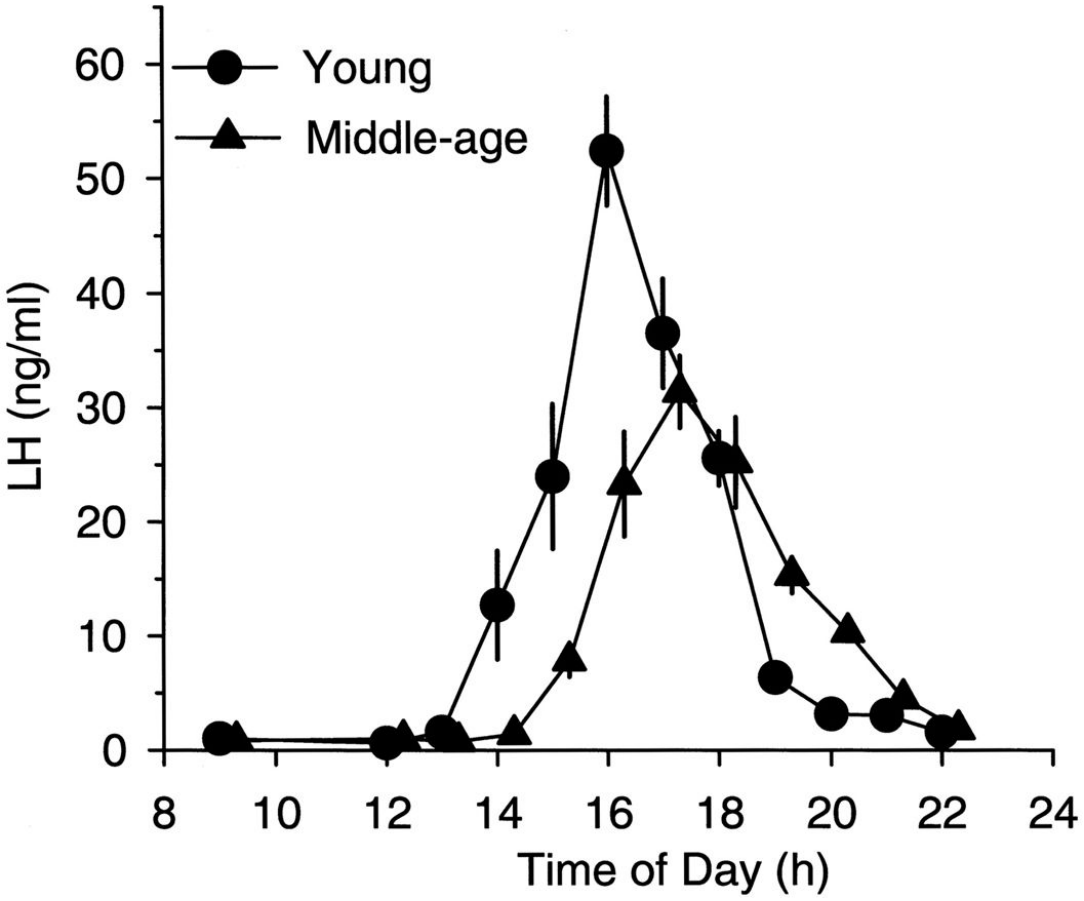

FIG. 1.

The luteinizing hormone (LH) surge is blunted and delayed in middle-aged, compared to young, rats. Young and middle-aged regularly cycling rats were sequentially bled from right atrial cannulae during the day of proestrus. Plasma was radioimmunoassayed for LH. The first significant increase in LH was delayed by 1 hour and attenuated significantly in middle-aged rats. [Reprinted with permission from Wise, P. M., 1982. Alterations in proestrous LH, FSH, and prolactin surges in middle-aged rats. Proc Soc Exp Biol Med 169(3), 348-354.]

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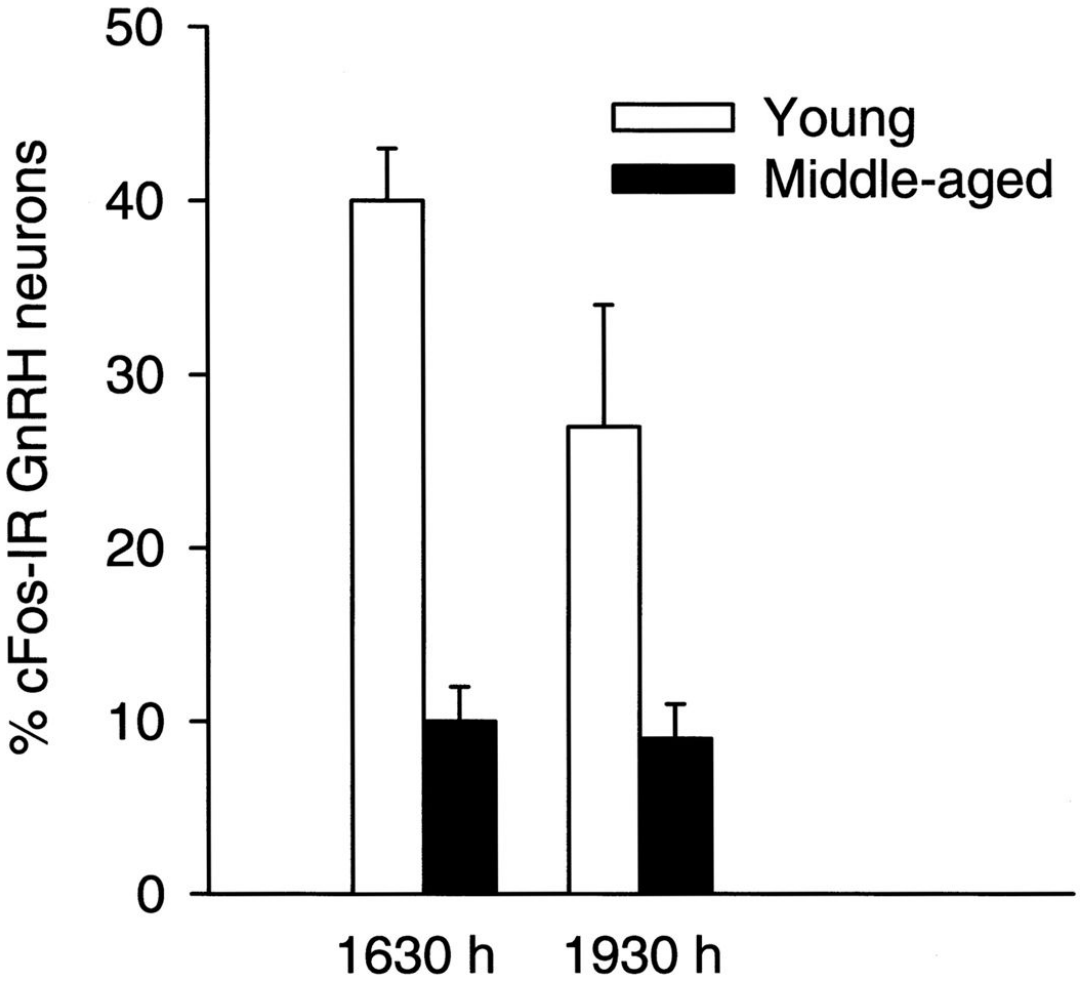

FIG. 2.

Percentage of gonadotropin-releasing hormone (GnRH) neurons that express Fos during the proestrous LH surge decreases with age. Young and middle-aged regularly cycling rats were perfused with paraformaldehyde and their brains sectioned for dual immunocytochemical localization of GnRH and Fos. Age significantly decreased the level of activation of GnRH neurons. [Reprinted with permission from Lloyd JM, Hoffman GE, Wise PM 1994 Decline in immediate early gene expression in gonadotropin-releasing hormone neurons during proestrus in regularly cycling, middle-aged rats. Endocrinology 134:1800–1805. Copyright The Endocrine Society.]

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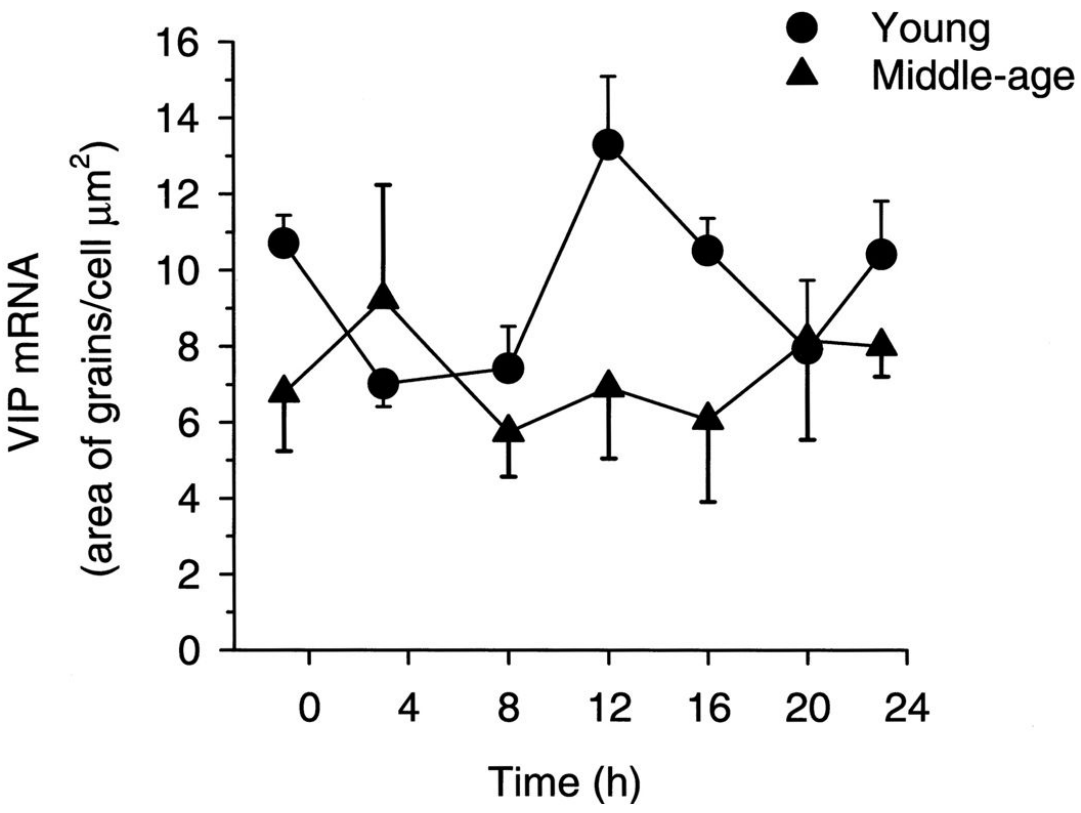

FIG. 3.

VIP mRNA levels/cell in young and middle-aged ovariectomized, estradiol-treated rats as measured by *in situ* hybridization exhibits age-related changes in rhythmicity. Young, middle-aged, and old rats were killed at 7 times of day over a 24-hour period. Young rats exhibited a diurnal rhythm in gene expression. The rhythm was no longer detectable in middle-aged or old rats. [Reprinted from Krajnak K, Kashon ML, Rosewell KL, Wise PM 1998 Aging alters the rhythmic expression of vasoactive intestinal polypeptide mRNA, but not arginine vasopressin mRNA in the suprachiasmatic nuclei of female rats. J Neurosci 18:4767–4774.]

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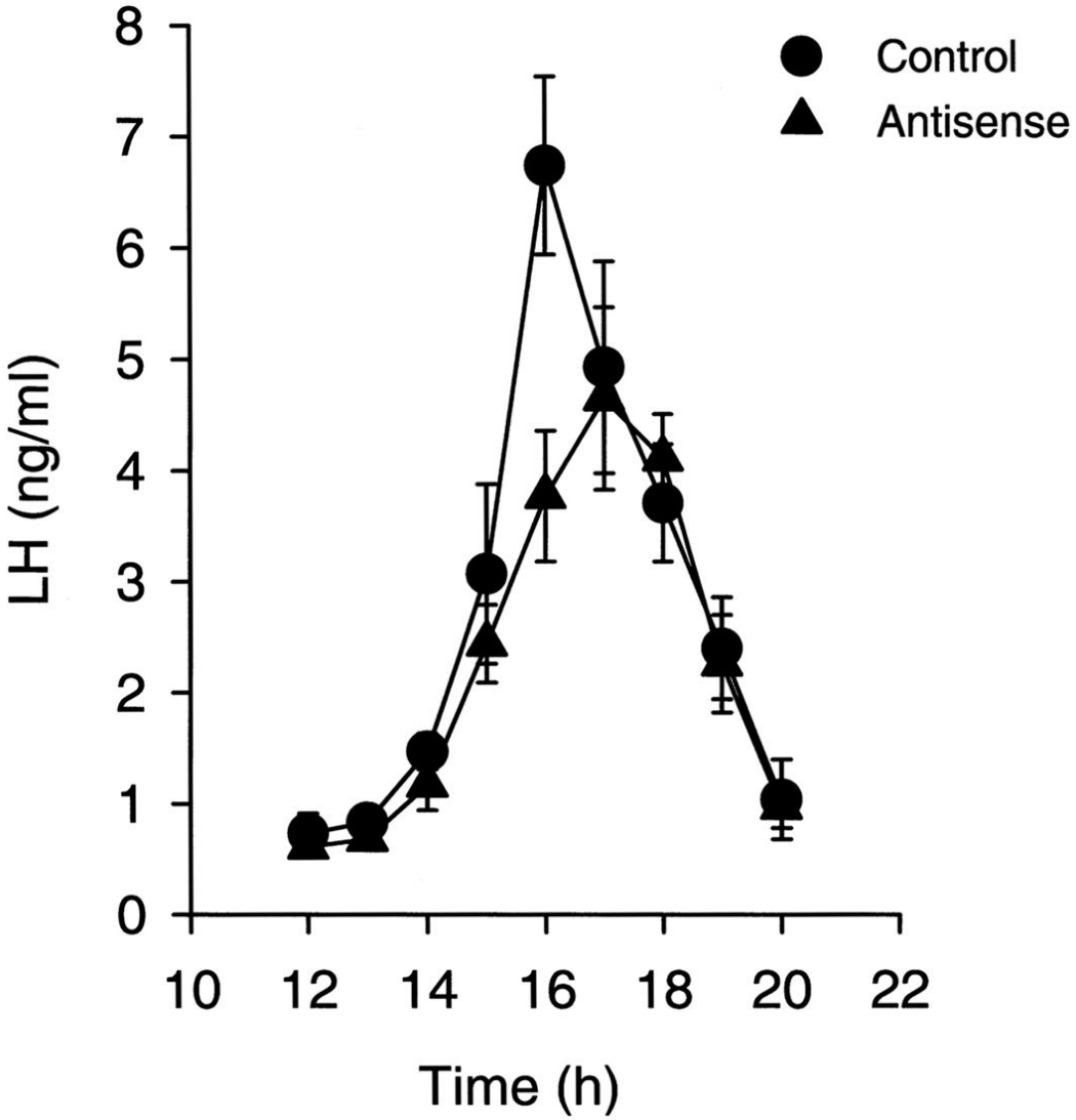

FIG. 4.

The steroid-induced LH is blunted and delayed in rats that were treated with antisense oligos to VIP or control scrambled oligos directly at the suprachiasmatic nucleus (SCN). Ovariectomized, estradiol-treated young rats were administered antisense or scrambled oligos and sequentially bled. The steroid-induced surge of LH exhibited changes that are remarkably like those observed during aging. [Reprinted with permission from Harney JP, Scarbrough K, Rosewell KL, Wise PM 1996 *In vivo* antisense antagonism of vasoactive intestinal peptide in the suprachiasmatic nucleus causes aging-like changes in the estradiol-induced LH and prolactin surge. Endocrinology 137:3696–3701. Copyright The Endocrine Society.]

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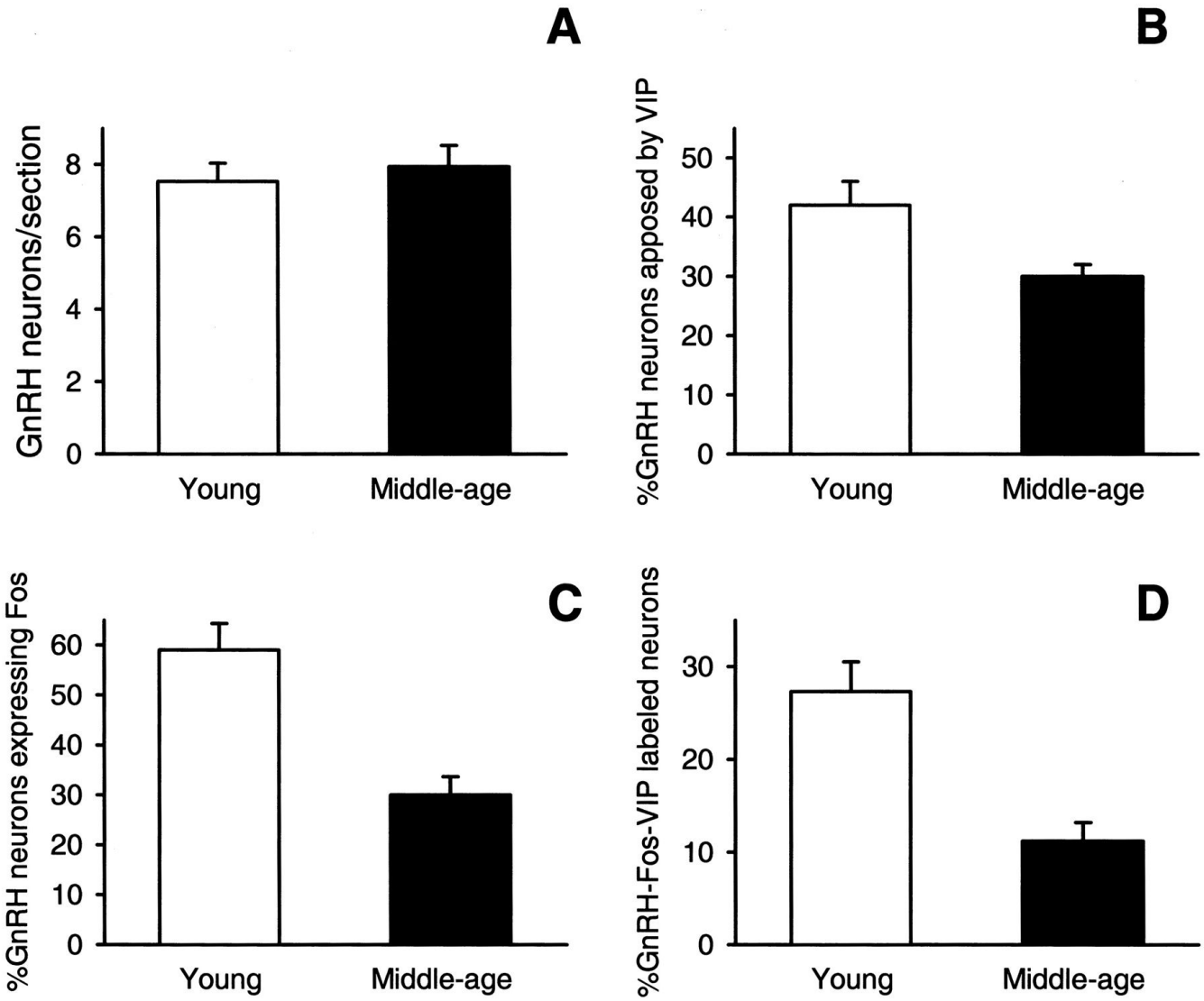

FIG. 5.

(A) Number of GnRH-immunopositive neurons per section; (B) percent of GnRH and VIP-immunopositive neurons; (C) percent of GnRH and Fos-immunopositive neurons; and (D) percent of GnRH, Fos, and VIP immunoreactive neurons in the preoptic area of young and middle-aged females during the peak of a steroid-induced LH surge exhibit age-related changes. Aging is associated with no change in the number of GnRH immunopositive neurons or the percent of GnRH and VIP immunopositive neurons. However, percent of activated GnRH neurons and the percent of activated GnRH that were closely apposed to VIP neurons decreased with age. [Reprinted from Krajnak K, Rosewell KL, Wise PM 2001 Fos-induction in gonadotropin-releasing hormone neurons receiving vasoactive intestinal polypeptide innervation is reduced in middle-aged female rats. Biol Reprod 64:1160–1164.]

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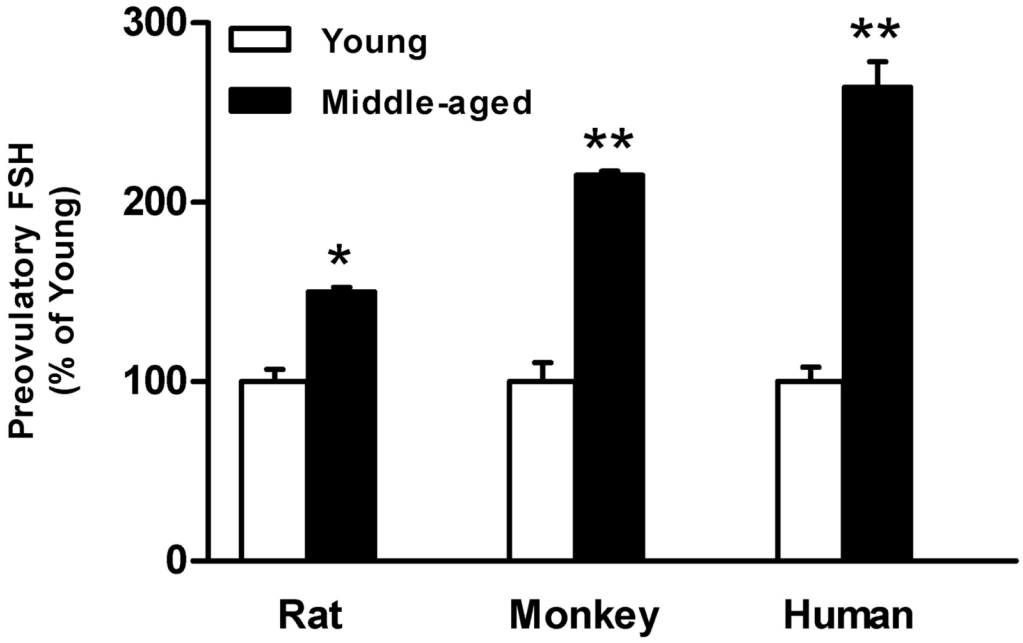

FIG. 6.

The preovulatory rise in circulating FSH is significantly elevated across species during middle-age prior to the onset of irregular reproductive cycles. The marked increase in plasma FSH during middle-age, which is a commonality shared between rats, monkeys and human females, is one of the earliest observable neuroendocrine markers indicative of impending reproductive decline. Mean group FSH values are expressed as percent of young ± SEM. *$p<0.05$, **$p<0.01$. [Adapted from: DePaolo, L. V. and Chappel S. C., 1986.Alterations in the secretion and production of follicle-stimulating hormone precede age-related lengthening of estrous cycles in rats. Endocrinology 118(3), 1127-1133, Downs, J. L. and Urbanski, H. F., 2006. Neuroendocrine changes in the aging reproductive axis of female rhesus macaques (*Macaca mulatta*). Biol Reprod 75(4), 539-546, and Reyes, F. I., Winter, J. S. and Faiman, C., 1977. Pituitary-ovarian relationships preceding the menopause. I. A cross-sectional study of serum follice-stimulating hormone, luteinizing hormone, prolactin, estradiol, and progesterone levels. Am J Obstet Gynecol 129(5), 557-564.]