REVIEW ARTICLE

Neuroendocrinology and ovarian aging

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The ovarian aging, a dynamic process that precedes the clinical manifestations of menopause, can be assessed using ovarian reserve biomarkers. It is well-known that reproduction during the later years of reproductive life has known limitations that challenge the success of assisted reproduction. Therefore, a review of the neuroendocrine modifications during this critical period of reproductive life may help to elucidate the ovarian aging process and its impact on reproduction. In this review, we aim to further the discussion of neuroendocrine changes taking place during the ovarian aging process that may impact reproductive function.

Keywords: Neuroendocrinology, ovarian aging, ovarian reserve, perimenopause

Introduction

Current epidemiological data support the critical role of age on female fecundity. The age at first childbirth has continued to rise since the beginning of the 20th century. Considering their significant effects on female reproductive life, the clinical presentation, physiology and molecular endocrinology of both normal and abnormal follicular attrition continue to gain importance in the field of endocrinology research. In particular, ovarian aging assessment has become a major challenge for older women attempting to become pregnant [1]. The identification of subjects with poor prognostic IVF success currently relies on ovarian reserve quantity markers including follicle-stimulating hormone (FSH), antimit-lerian hormone (AMH), inhibin B, and antral follicular count (AFC) [2]. The accurate prediction of poor or high responders has opened avenues for adapted management, although definitive data concerning efficacy are not yet available. Interestingly, similar tests for ovarian reserve function have been used to understand the endocrine changes occurring during menopausal transition. Recently published data describe interrelationships in hormone levels, including inhibin B, inhibin A, E₂, AMH, and FSH, throughout reproductive age [3]. Ovarian reserve function tests have been used also to differentiate between the impacts of hormonal changes and those of the aging process. This brief review will discuss the normal interrelationship of pituitary–ovarian hormones in fertile women and the impact of the aging process on this relationship, particularly during the menopausal transition.

GnRH pulses and gonadotropin secretion

A pattern of pulsatile gonadotropin-releasing hormone (GnRH) secretion is required to sustain gonadotropin synthesis and secretion. The frequency and amplitude of GnRH pulses determine the expression and secretion of pituitary luteinizing hormone (LH) and FSH-β. Studies in rodents have demonstrated that rapid frequency GnRH pulses, i.e., >1 pulse per hour, favor LH secretion, whereas slower frequencies favor FSH synthesis and secretion [4]. Importantly, there is an interrelationship between the ovarian and pituitary hormones in the ovulatory menstrual cycles that change over the course of woman’s reproductive life. Interestingly, aberrant pulsatility of GnRH modifies this interrelationship during the perimenopausal period as well as during periods of hypothalamic ovarian dysfunction, including cases of hypothalamic amenorrhea or polycystic ovary syndrome (PCOS) [5]. Differential expression of FSH and LH is regulated in part by direct ovarian feedback on the pituitary. Estradiol (E₂) levels rise gradually during the follicular phase under the influence of FSH, reaching a preovulatory peak that promotes LH release (i.e., the midcycle LH peak). LH levels subsequently undergo a second rise and fall during the luteal phase, representing secretion from the corpus luteum.

The inhibins include two isoforms, B and A. Inhibin B is secreted by the granulosa cells and mirrors the pattern of FSH secretion during the follicular phase. Both inhibin B and E₂ are the principal feedback regulators of FSH secretion. Inhibin A levels remain at consistently low levels throughout the follicular phase of the cycle and show a similar pattern of expression to that of progesterone (P₄) during the secretory phase [3].

During normal ovulatory cycles, increasing GnRH frequency during the follicular phase favors LH synthesis prior to the LH surge. Following ovulation, P₄ decreases the frequency of GnRH pulses, thereby favoring FSH synthesis during the luteal transition of a nonconceptive cycle. Therefore, changing frequencies of GnRH stimulation on the gonadotrophs is one of the mechanisms driving differential gonadotropin secretion [5].

Luteal P₄ biosynthesis largely depends upon the action of pituitary-derived LH stimulating the cyclic adenosine monophosphate (AMP) second messenger signaling system, which serves to regulate genes essential to hormonal synthesis and luteal development. Two critical endocrine events support P₄ secretion in primate corpus luteum (CL) physiology: (1) The LH surge is the signal for follicular rupture and the luteinization of theca and granulosa cells, and (2) LH pulses during the luteal phase are critical to the development and function of the CL [6]. The classical work of Filicori et al. [7] demonstrated a progressive slowing of LH pulses during the luteal phase, with the mean LH pulse frequency declining from 15.2 pulses/24 hours in the early luteal phase to 8.4 pulses/24 hours in the late luteal phase. A trend towards a reduction in the amplitude

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of LH pulses was also observed. Interestingly, the decreasing frequency of all pulses correlates with the day of the luteal phase but not with the plasma \( P_4 \) levels. During the early luteal phase, the plasma levels of \( P_4 \) evaluated over 24 hours did not show any relationship to the episodic LH release, which may suggest a variant response at the level of the LH receptor in the steroidogenic luteal cells [8]. Conversely, during the mid- and late-luteal phases, plasma \( P_4 \) does correlate with episodic LH release. These data indicate the importance of the gonadal receptiveness to gonadotropin. On the other hand, there is evidence that the LH pulse frequency changes with reproductive age. Reame et al. [9] showed an increase in the luteal phase LH pulse in women during their forties. In contrast, Matt et al. [10] have reported decreased LH pulse amplitudes and frequencies in the midfollicular phase of the reproductive cycles of late reproductive-age women. This finding implies that women may exhibit enhanced sensitivity to the negative feedback of midfollicular \( E_2 \) on their hypothalamic–pituitary axis or that midfollicular-phase \( E_2 \) is elevated in women during their menopausal transition.

**The ovarian aging process**

The reproductive aging process comprises a gradual decrease in both the quantity and the quality of the oocytes residing within the follicles present in the ovarian cortex. It is thought that each woman receives an endowment of oocytes during fetal development [2]. At the fourth month of fetal development, ovaries contain approximately 6–7 million oocytes, each surrounded by a layer of flat granulosa cells forming the primordial follicle pool. However, at birth only 1–2 million primordial follicles remain. After birth, follicular apoptosis decreases, and at menarche at least 300,000 to 400,000 follicles remain. During the reproductive years, the continued and gradually accelerated decline of oocytes causes the number of oocytes to drop below 10,000 by the time of menopause.

Concomitant with the reduction in follicular number and quality, a number of biological and social-demographic events occur. The loss of oocyte competence is presumably due to an increasing rate of meiotic nondisjunction, which results in an increasing rate of aneuploidy in early embryos as maternal age increases [11]. It is also possible that changes in the quality of the granulosa cells surrounding the oocyte could affect the oocyte competence [12].

These biological events clearly decrease the monthly fecundity of couples, thereby influencing natural fertility and population replacement in wealthy western societies with the tendency to postpone fertility.

In 2011, the Stages of Reproductive Aging Workshop (STRAW) proposed the following definition [13]: Menopause is the permanent cessation of menstruation due to the loss of ovarian follicular development. It regularly occurs after 1 year of amenorrhea.

Perimenopause is the period from immediately before menopause, when the endocrinological, biological, and clinical features of approaching menopause initiate, to 1 year after menopause.

The menopausal transition period is associated with the clinical signs of advancement in the reproductive aging process. Variable cycle lengths (>7 days different form normal or >2 skipped cycles) and an interval period of amenorrhea have been proposed as a clinical definition of the menopausal transition period.

Neurosteroids are steroids synthesized in neuronal cells independent of ovarian and adrenal contributions. Their importance for brain function has been demonstrated using animal models. During the ovarian aging process, the decreasing levels of the circulating steroids are not necessary reflected in the neurosteroid concentration. Whether compensatory production of neurosteroids plays a role in the neuroendocrine changes during ovarian aging process remains to be determined [14].

**Assessment of ovarian reserves**

The clinical and biochemical ability to predict ovarian reserve and ovarian function across a woman’s life remains an important goal in clinical and basic research focused on fertility preservation, assisted reproduction techniques (ART), and fertility counseling. The continuous quantitative and qualitative losses of follicles and oocytes have been recognized as critical factor in reproductive dysfunction [15]. However, the availability of different ovarian stimulation protocols as fertility options has generated the need for individual assessments of ovarian potential in healthy and disease states.

**Baseline FSH**

Baseline FSH levels have been used for many years to predict a woman’s ovarian reserve, her response to ovulation induction, and her potential success with IVF [16]. Accurately determining FSH levels, however, has many difficulties. One issue is the inconvenience of required blood draws on days 2 or 3 of menses. A second issue of concern is the degree of cycle-to-cycle fluctuation in baseline FSH levels, which may be at least partially caused by the dependency of FSH levels on the negative feedback of \( E_2 \) and inhibin levels [3]. These interrelationships between the pituitary and the ovary make the FSH test an indirect and less sensitive ovarian marker.

**Inhibin-B**

The inhibins are dimeric polypeptides and include two isoforms A and B both secreted by the granulosa cells. Inhibin B is secreted largely during the follicular phase by the growing follicles and may have paracrine functions that affect testosterone production and folliculogenesis. Conversely, inhibin A is mainly secreted by the luteal cells and has the ability to suppress FSH secretion. Early studies suggest an association between a diminished ovarian response, lower pregnancy rates, and reduced inhibin B levels. However, more recent studies indicate that inhibin B levels are a more reliable indicator of ovarian activity than of ovarian reserve [17].

**The antral follicle count**

Ultrasoundographic imaging is an effective, easy-to-use, safe, and readily available noninvasive means to evaluate fertility potential. Ultrasonography is routinely used to evaluate ovarian follicle number as a means to estimate the ovarian reserve in women. Normal AFC has been defined as the total number of antral follicles 2–10 mm in size per ovary \((n \geq 4)\). A marked reduction in the number of antral follicles per ovary \((n = 2)\) and a change in the ovarian volume resulting from a decline in the number of follicles may raise the suspicion of or confirm early ovarian failure or a poor response to ovarian stimulation [18]. AFC may be estimated at specific times of the menstrual cycle (days 2–5) and are correlated to FSH levels. As women age, there is a gradual decline in the number of ovarian follicles and their responsiveness to gonadotropin stimulation, which in turn determines the true size of the follicular cohort that is responsive to exogenous FSH. There is a wide individual variation in the ovarian response to exogenous gonadotropin.
stimulation that occurs in women of advanced reproductive age [19].

It remains unclear whether the probability of oocyte fertilization and pregnancy can be estimated by assessing the ovarian reserve with AFC. The capacity of an oocyte to be fertilized in older women has not been predicted solely by the age-related decline in the number of antral follicles. Some young women with low AFC and poor responsiveness to stimulation have higher IVF conception rates than the rate of conception seen in older poor-responders. A lower age-related risk for aneuploidy may contribute to the higher IVF conception rate observed in younger poor-responders. While low AFC signal a poorer response to ovarian stimulation, additional predictors of ovarian reserve are needed to identify which oocyte has the capacity to be fertilized and progress to clinical pregnancy following ART [19].

**Antimullerian hormone**

AMH is a dimeric glycoprotein belonging to the transforming growth factor β family and is exclusively produced by granulosa cells of preantral follicles. Production of AMH starts in the preantral follicle and continues in the antral follicles. It is thought that AMH has an autocrine–paracrine action on follicular development. Follicles stop producing AMH when they become a dominant follicle. The number of small antral follicles is directly related to the total size of the primordial follicle pool. These characteristics of AMH expression make this peptide one of the most reliable markers of ovarian reserve. Interestingly, AMH serum levels do differentiate between women undergoing imminent ovarian failure or full premature ovarian failure and women with PCOS at risk for hyperstimulation. These data suggest that the combination of pre-AFC and AMH levels are currently the best assessment of ovarian reserve in both healthy and diseased women [20].

**Endocrine changes during menopausal transition**

The STRAW workshop (2001) proposed the following stages of reproductive aging:

- **STRAW 4**: Mid reproductive age, regular cycle
- **STRAW 3**: Late reproductive age, 40–45 years of age, regular cycle with raised FSH levels
- **STRAW 2**: Equivalent to stage 3 but with irregular menstrual cycles
- **STRAW 1**: Equivalent to the late menopausal transition

Recently, investigators from Australia characterized the hormonal changes associated with the menopausal transition in relation to the classic description of hormonal changes occurring in late reproductive life [21,22]. Standard endocrine studies assessing FSH levels during the transition period postulated the existence of inhibin B to explain the observed increase in FSH levels during the transition period. It was concluded that inhibin B is the principal ovarian player regulating FSH, whereas steroids exert their actions in both positive and negative fashions primarily on LH. Antimullerian hormone (AMH) is negatively associated with FSH, but it does not contribute to its feedback regulation [3].

Interestingly, luteal phase endocrinology does not regularly change in women with regular menstrual cycles during the menopausal transition. Plasma levels of E₂, P₄, and inhibin A are unaltered compared with young women [23]. However, in the clinical setting, women of older reproductive age exhibit increased FSH levels during the luteal–follicular transition, which results in the advancement of normal dominant follicular growth with a shorter follicular phase, CL dysfunction, and changes in cycle length. Furthermore, the elevated follicular FSH levels will often support the follicular development of more than one dominant follicle, resulting in higher follicular E₂ levels and an increased occurrence of dizygotic twinning.

**Conclusions**

Ovarian insufficiency is a progressive process beginning years before the cessation of menstrual flow in normal women. Although it has been considered a physiologic consequence of aging, a number of women are also affected by nonphysiologic reductions in their ovarian reserve regardless age. Unfortunately, the early prediction of these patients is limited. It is thought that ovarian feedback dictates the neuroendocrine control of ovarian cyclical function. The data reviewed here indicate that the progression of STRAW stages is associated with the increased levels of FSH and LH, the variable levels of E₂, and the decreased levels of P₄ during the luteal phase. The key role of inhibin B in regulating FSH levels and the role of the E₂/P₄ ratio in regulating the FSH/LH ratio are also supported. The significant reduction of inhibin B and AMH levels might be useful in predicting STRAW stages. However, in this context, the signs and symptoms of ovarian insufficiency should be supported, and the majority of the time, the diagnosis of perimenopause can be made on clinical grounds.

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**References**


