REPRODUCTIVE TESTS IN FEMALES

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OVERVIEW

In normal females the hypothalamic-pituitary-ovarian axis undergoes major changes during the lifespan so that plasma levels of gonadotropins and ovarian steroids vary dramatically and must be interpreted according to the time of life and the patient's clinical circumstances. Surprisingly, perhaps, the axis is activated at birth and becomes quiescent during the first year, remaining so until the onset of puberty. During the reproductive years from menarche until menopause, relatively regular menstrual cycles are the norm, except during pregnancy and lactation. During the perimenopausal transition, cycle length and corresponding hormone values are less predictable, while after complete cessation of menses, the hormonal pattern reflects ovarian failure.

As a result of these variable physiologic states, the history is the most important initial investigation into possible reproductive abnormalities. Depending on the presenting features, it is important to inquire about the timing of puberty-in terms of the consistency of its signs and the tempo of progression, and the characteristics of the menstrual cycle. Typically, cycles vary in length at the extremes of the reproductive age span after menarche and just before menopause, reflecting anovulatory cycles (1). The inter-menstrual interval is most consistent from age 20 – 35. Each woman, however, tends to have her own individual pattern-some are "always" regular and other have variability within the intervals considered to represent ovulatory cycles (roughly 23 – 34 days). Still others have an oligomenorrheic pattern that probably reflects underlying dysfunction. A change in the woman's usual pattern of menses may be an important clue to a disorder-or to a physiologic state such as pregnancy. Additional questions should be directed to a history of pregnancy, or attempted pregnancy, and lactation.

Physical examination may be quite revealing when the disorder reflects a change from the norm-such as delayed or early puberty, or hirsutism. In these cases, comparison with well-established normative features is essential. In other settings, such as recent onset amenorrhea, physical examination (apart from features of pregnancy) may not be very helpful.

Ultimately, laboratory tests help to fill in the gaps and questions raised by the history and physical examination. Measurement of basal hormone levels provides important information but must be interpreted in the clinical context. Less commonly, provocative tests of the hypothalamic-pituitary-ovarian axis help to establish a diagnosis.

MEASUREMENT OF BASAL HORMONE LEVELS

Gonadotropins (luteinizing hormone (LH), follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG)) and the major steroid products of the ovary (estradiol and progesterone) are the most useful baseline tests for the evaluation of reproductive function. Clearly, there also is a role for measurement of thyroid hormones, androgens, glucocorticoids and prolactin (covered elsewhere in this text), as abnormalities of these hormones may alter reproductive function. When interpreting the results of these tests, it is critical to understand the vagaries of steroid and glycoprotein secretion and assays. Estradiol, progesterone and LH are secreted in a pulsatile fashion so that a single measurement may not reflect the overall physiology of the patient. Thus, while high values can be taken at face value, a single low value does not exclude the possibility of higher concentrations, as the sample may represent the nadir between pulses. By contrast, plasma FSH concentrations vary relatively little.

Assay methodology also may affect interpretation of the results. Estradiol circulates at nanomolar levels and is difficult to measure, especially at the lowest physiologic concentrations of 0 – 15 pg/mL. Thus, it is important to know the detection limit of the assay (and whether it was determined by the assay coefficient of variation), and the intra- and inter-assay coefficients of variation at different dose levels. Assays with a relatively high limit of detection and large coefficient of variation near the detection limit should be avoided when it is important to measure estradiol at low levels with accuracy.

Changes in glycoprotein assay methodology over the last 30 years have changed the “normal” range of LH and FSH. As these glycoproteins circulate as different isoforms, polyclonal antibodies may recognize a variety of isoforms, whereas monoclonal antibodies, used in two-site assays, may be more selective. Additionally, the use of chemiluminescent (ICMA) or fluoroluminescent (IFMA) rather than radioactive detection methods has decreased the limit of detection of these assays (2). Thus, both the threshold of detection and the normal range may differ between assays. For example, the normal follicular phase ranges extracted from various publications are shown in Table 1. As a result, when evaluating commercially available assays, it is important to choose one that has been validated in these ways by the company or investigators, in the clinical population of interest. Additionally, the antibodies in some LH assays do not recognize variant forms of the peptide structure, and may underestimate the circulating value by up to 70% (3).

Table 1. Comparison of follicular phase normal ranges for LH and FSH as judged by different immunoassays.

Assay Type	LH (IU/L)	FSH (IU/L)
RIA (4)	10 – 31	6 – 17
RIA (5)	5 – 25	3.2 – 9

ICMA (6,7)	2 – 9	1.8 – 11.2

During infancy, plasma LH and FSH concentrations may overlap with values seen in adults, and estradiol levels may be increased in comparison to normal pre-pubertal values. However, by age 1 – 2, this neonatal activation of the axis diminishes (6 – 9). Thereafter before puberty, serum estradiol concentrations are generally less than 10 pg/mL. Plasma LH is generally undetectable by radioimmunoassay, and less than 0.15 IU/L when measured in a more sensitive assay. By contrast, plasma FSH is generally detectable by all assays.

The normal range for LH increases at each stage of puberty; importantly, the lower limit of normal increases so that an increased basal level can be used to diagnose precocious puberty (10,11). While the highest limit of normal FSH also increases over puberty, the lowest normal value does not change very much, limiting its utility for the diagnosis of puberty.

During the reproductive years these hormones have a characteristic pattern based on the menstrual cycle and pregnancy (Figures 1 and 2). As can be seen by the value scales, circulating estradiol and progesterone values increase signficantly during pregnancy compared to the menstrual cycle. Both LH and FSH levels are suppressed in pregnancy compared to menstrual cycle values (12,13) if assays that discriminate LH from hCG are used. Other assays have significant cross-reactivity between the two glycoproteins and may give falsely increased LH values during pregnancy, or falsely elevated hCG levels during menopause when LH concentrations increase. It is important to know the cross-reactivity of these compounds when interpreting results in these settings.

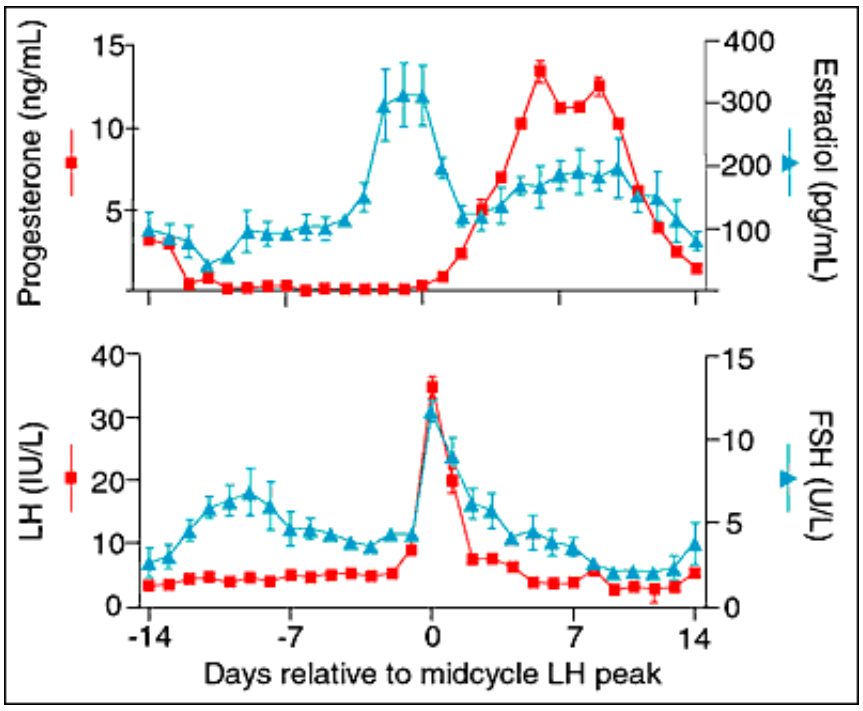

Figure 1.Patterns of LH, FSH, progesterone and estradiol during the normal menstrual cycle, normalized to the LH peak.Adapted from reference 22 with permission.

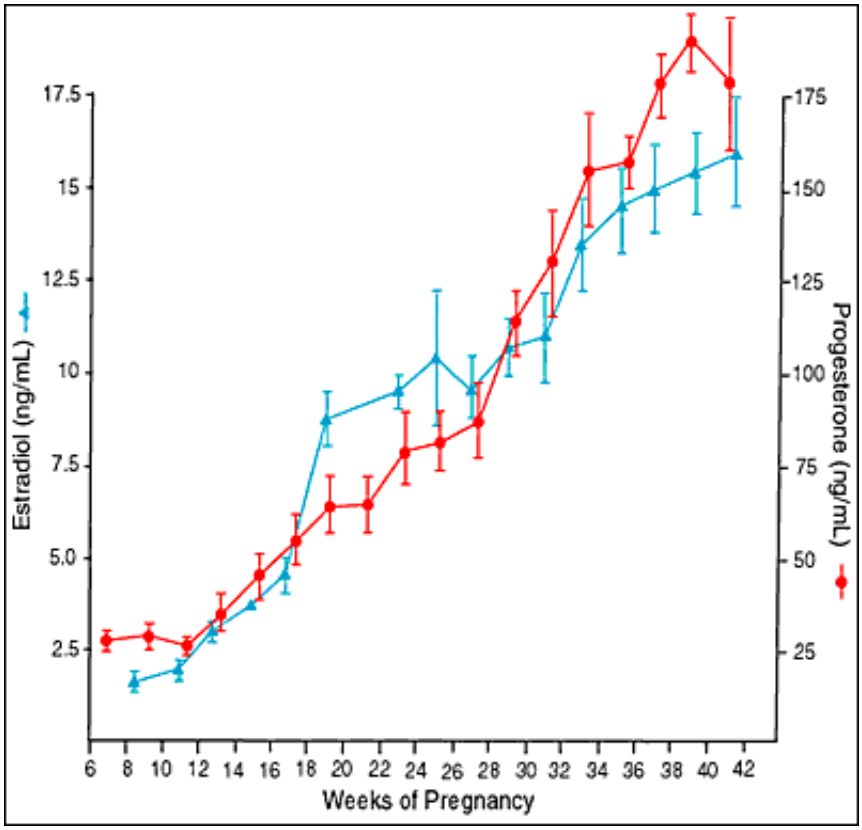

Figure 2.Pattern of serum estradiol and progesterone values during normal pregnancy.Adapted from ref. 32 with permission.

Measurement of basal hormone levels is useful for the diagnosis of some but not all reproductive abnormalities when values differ from the age-adjusted normal range (Table 2).

Table 2. Utility of basal hormone measurements in various disorders

Condition	Hormone and interpretation	Caveats
Precocious thelarche	estradiol > 13.6 pg/mL	Estradiol may be in this range in normal infants during the first few months of life, after which values are generally normal. The response to LHRH may be more helpful to distinguish between isolated precocious thelarche and precocious puberty (14).
Precocious Puberty	LH > 0.6 IU/L in ultrasensitive assay (IFMA)	This has a 63% sensitivity and 100% specificity for the diagnosis of gonadotropin-

		dependent precocious puberty in girls (10,11).
Primary Gonadal Failure	LH and FSH greater than age-adjusted normative values	In children, requires a highly sensitive assay (15); FSH is more clearly abnormal than LH. In adults, FSH is more sensitive also and RIA can be used.
Premature Ovarian Failure	FSH > 40 IU/mL on two occasions, at least one month apart in a woman with amenorrhea for at least six months, aged <40	These women may have intermittent ovarian function during which gonadotropin and estradiol concentrations may be normal. Thus, arbitrarily, two abnormal values are required for this diagnosis.
Amenorrhea	LH, FSH	Increased values (> 40 mIU/mL) suggest primary ovarian disorders or menopause; normal and diminished values are found in other conditions (5).
Pregnancy	hCG	Urine test strips and some serum assays do not distinguish between LH and hCG. If such assays are used, states of LH-excess (menopause, LH surge) should be excluded.
Luteal Phase	Progesterone > 2 ng/mL	Progesterone is pulsatile and may be below this value in a woman with a normal luteal phase. Values > 2 ng/mL indicate corpus luteum presence; values > 5 – 7 ng/mL are “normal”

DYNAMIC STIMULATION AND OTHER PROVOCATIVE TESTS OF THE HYPOTHALAMIC-PITUITARY-OVARIAN AXIS

Progesterone withdrawal

What is it?

This test examines whether the endometrium has been primed with estrogen sufficiently to respond to progesterone with withdrawal bleeding when progesterone levels fall. A normal response (bleeding) requires adequate previous estrogen exposure, and a normal uterus and outflow tract. As a result, lack of response requires documentation of normal anatomy before a diagnosis of hypoestrogenism can be inferred. The presence or absence of a response does not correlate well with serum estradiol levels at the initiation of treatment, perhaps because of the pulsatile nature of serum estradiol concentrations. By contrast, endometrial thickness on ultrasound of more than 1.5 mm predicts those women who respond to progesterone. However, this is not absolute, as occasionally a woman with a thicker lining will not respond (16-18).

How is it done and interpreted?

Progesterone is given in a variety of ways: as progesterone in oil, 100 – 200 mg im, or as medroxyprogesterone acetate, 10 mg daily for 5 – 12 days. The woman is instructed to expect bleeding per vagina beginning 1 – 14 days after the last dose (or the injection). Any amount of spotting or bleeding is considered a positive test.

When is this helpful?

1. Because the test is a bioassay of estrogen exposure, it reflects the extent of hypoestrogenism in women with amenorrhea. While it is thought that a response to the test indicates “adequate” estrogen levels to prevent osteopenia, there is a poor correlation between the estradiol level at the time of the treatment initiation, and the response. However, the response to progesterone currently is not used to gauge the need for estrogen replacement therapy.
2. Because the test is a bioassay of estrogen exposure, it reflects the extent of endometrial estrogen exposure in postmenopausal women regardless of exogenous estrogen treatment. In one small study, 3 of 5 postmenopausal women with a positive test had endometrial hyperplasia, while none of 25 women with a negative response had abnormal endometrial pathology (19).
3. Because the test evaluates the integrity of the uterine-vaginal outflow tract, lack of a response may identify women with disorders of this system, such as Ashermann syndrome, hypoplastic or absent uterus or imperforate hymen.

Is there a better test?

Physical examination and ultrasound examination of the vagina, cervix and uterus provide a more direct way to evaluate uterine and vaginal anatomy. As a result, the progesterone challenge test currently is more a test of historical interest rather than a routinely standard test.

GNRH STIMULATION FOR ASSESSMENT OF PITUITARY RESERVE

What is it?

The GnRH stimulation test evaluates the ability of gonadotropes to secrete LH and FSH after exposure to the natural hypothalamic releasing hormone, GnRH, or an analog.

How is it done and interpreted?

GnRH (100 ucg) is administered intravenously or subcutaneously and plasma LH and FSH are measured at 0, 15, 30, 45 and 60 minutes.

Normal children: the response varies according to the stage of puberty. Typically, the FSH peak is higher than the LH peak in pre-pubertal girls, and at Tanner stage II. In fact the maximal FSH peak in pre-pubertal girls exceeds the upper limit of the response in women. Beginning at Tanner II, and continuing through Tanner stage V, the FSH peak responses of girls are similar to those of women (roughly 5 – 12 IU/L). In contrast to FSH, at Tanner I pubertal stage, the LH peak is below that of adult women (roughly 8 – 32). However, some children overlap the adult range at Tanner II and some may exceed the adult range at Tanner IV or V. At Tanner III stage and above, the LH peak is usually greater than the FSH peak (10,11).

Girls with precocious central (gonadotropin-dependent) precocious puberty: These patients show baseline LH (Table 1) and peak LH values after GnRH that are greater than prepubertal norms; FSH basal values generally are also increased, but peak values may be within the prepubertal range (2, 9,10). The cut-off points used as diagnostic criteria vary between studies and LH assays. One recent evalution used an IFMA assay with an LH detection limit of 0.6 IU/L and found a sensitivity of 63% and specificity of 100% for the detection of central precocious puberty using a basal LH cut-off value of 0.6 IU/L or more. The corresponding sensitivity was greater (92%) at 100% specificity for the peak LH value after GnRH, using a cut-off value of 6.9 IU/L. The abnormalities in basal and peak gonadotropin responses normalize in girls who are treated effectively with LHRH analog.

Thelarche: The mean basal plasma LH levels and the peak LH response to LHRH stimulation were significantly less in girls with isolated thelarche than in girls with complete sexual development. The mean basal plasma FSH levels did not differ between these groups, but the peak FSH response to LHRH was greater in girls with isolated thelarche than in girls with complete sexual development (14,20).

In normal premenopausal adult females: The GnRH-stimulated peak plasma LH concentrations (measured by IFMA assay) range from 7.6-31.7 IU/L and peak FSH concentrations from 4.6 to 11.7 IU/L in these women (11). Women with hypogonadotropic hypogonadism may have blunted responses, but may overlap the normal range (5).

When is it useful?

The LHRH test is useful to monitor adequate treatement of gonadotropin-dependent precocious puberty, and may be helpful in establishing the diagnosis of this condition, although often the baseline plasma LH concentration is sufficient, if measured in a sensitive assay. The LHRH test, in general, is not a primary diagnostic test in other settings, although it may provide useful adjunctive information.

Is there a better test?

While it was extensively evaluated for the discrimination of disorders of precocious or delayed puberty and amenorrhea, today the GnRH test has been supplanted by measurement of basal FSH and/or LH using very sensitive sandwich assays.

TESTS OF "OVARIAN RESERVE"

Reproductive aging in women occurs gradually: fertility is greatest in the third decade, diminishes in the fourth, and ceases sometime in the fifth, generally before the time of menopause (21). This process is thought to reflect both a decrease in both the quality and quantity of eggs available for recruitment during each cycle. There is great interest in development of tests to predict the fecundity of an individual woman; these tests have been termed tests of ovarian reserve.

FSH increases greatly at the time of menopause (see above), presumably because of decreased negative feedback as a result of diminished ovarian production of estrogens and inhibins. This observation led to investigation of the ability of basal FSH-or the FSH response to altered estrogen feedback-to predict pregnancy in women undergoing in vitro fertilization (IVF). A more direct evaluation of ovarian function may be obtained by measuring estradiol production after GnRH agonist administration, or by quantifying the number of small follicles detected by ovarian ultrasound. All of these measures would be expected to decline as the number of small follicles decreases. Because there is no established "gold standard" test of ovarian function, most studies have used pregnancy as a surrogate outcome measure of normal function.

What is it?

Plasma FSH concentrations are lowest at the luteal-follicular transition (22); as women age, FSH values increase at this time.

How is it done and interpreted?

The test involves measurement of plasma FSH on cycle day 1 – 3. Cut-off points for an abnormal value differ between centers, from 14.2 IU/mL to 26 for a single measurement, or 26 IU/mL for the sum of days 1 and 2. These differences are possibly related to the different assays used, but also to the arbitrary nature of the criterion, which is set independently by each group (23,24).

Values greater than the cut-off points indicate "diminished" ovarian reserve. Women with diminished ovarian reserve have increased rates of reproductive loss – as high as 71% in one study of women of all ages who became pregnant spontaneously or through assisted reproductive technologies (25). However, because it is clear that FSH values increase as women age, the contribution of ovarian age per se has been controversial. One study evaluated this issue by comparing IVF outcome in women less than 40 years old with abnormal FSH to that of women more than 40 years old with normal basal FSH. In that study, older women with

normal FSH had a better ovarian response to gonadotropin stimulation, and so had fewer cycles cancelled than did the younger women. However, when the younger women responded adequately, they had a better implantation rate per embryo than did the older women. On balance, the younger women had a higher ongoing pregnancy rate (per cycle of IVF) (24). These data support earlier conjecture that age is associated with diminishing egg quality, while increased FSH represents diminished number of follicles (25).

When is it useful?

Measurement of basal serum FSH concentrations may be useful to predict the success of IVF in women less than 40 years or those with a single ovary, and to evaluate the cause of infertility in those without a known cause. Thus it may be very useful when counseling a couple about ART choices (26). Some groups also use it to exclude younger women with abnormal values from attempting IVF. This may be reasonable if only a few cycles of IVF can be offered, because of the poor response rate of these women. However, the good rates of ongoing pregnancy when a cycle is successful (24) and the documentation of spontaneous pregnancies in this cohort (25) suggests that an increased FSH is not an absolute predictor of failure of pregnancy in these women. (However a very elevated value in a woman who has been amenorrheic suggests ovarian failure rather than diminished ovarian reserve, and would suggest that the chance of pregnancy is extremely low.)

Is there a better test?

Age alone (greater than 40 years) also predicts diminished quality of ova. In women aged less than 40 years, the clomiphene citrate challenge test or ovarian ultrasound may be alternative approaches if the basal FSH is normal. However, each is more expensive and less convenient than a single blood test.

CLOMIPHENE CITRATE CHALLENGE TEST

What is it?

This test relies on the ability of the pituitary gland to respond to decreased estrogen feedback by increasing FSH levels. Clomiphene citrate is a mixed estrogen agonist-antagonist with antagonistic effects at the pituitary gland, where it blocks the action of circulating estrogen. In the early follicular phase, inhibin B and estradiol levels are increasing as a result of growth of small follicles, and restrain the FSH increase after clomiphene citrate.

How is it done and interpreted?

Clomiphene citrate is given from day 5 to 9 of the cycle as a 100 mg oral dose. FSH is measured on days 3 and 10 (before and after the agent). An abnormally high value (cut-off points 10 – 26 mIU/mL in various studies) indicates diminished ovarian reserve and is interpreted just as a basal FSH test (23,27).

When is it useful?

The clomiphene citrate challenge test is useful in the same settings as the measurement of basal FSH. As it provides no additional information when the basal day 3 FSH is elevated, there is no reason to perform the test in those women.

Is there a better test?

This remains controversial and largely untested.

OVARIAN ULTRASOUND

What is it?

Ovarian ultrasound involves imaging of the ovaries with ultrasound, usually with a transvaginal probe. The ovarian length, depth, width and volume can be calculated using either two-dimensional or three-dimensional images. The number of antral follicles can be counted and blood flow can be estimated using Doppler measurements. Ovaries with fewer antral follicles have a smaller volume and less blood flow, correlating with diminished ovarian reserve.

How is it done and interpreted?

Arbitrary cut-off points have been set for normal premenopausal total ovarian volume (> 3 cc). Women with volumes less than this may have impaired pregnancy outcome, although volume is not an absolute predictor of IVF success (28,29).

When is it useful?

The test may be useful to predict the amount of gonadotropin needed for stimulation during IVF cycles and to predict the number of oocytes that will be retrieved. This test has not become a standard of practice.

Is there a better test?

Formal comparisons between age, the clomiphene citrate challenge test and ovarian volume must be made to determine the relative contributions of each test.

GNRH AGONIST STIMULATION

What is it?

This test relies on the ability of GnRH agonists to stimulate LH and FSH release and stimulate estradiol production.

How is it done and interpreted?

After GnRH agonist down regulation of pituitary LH and FSH secretion, women self-administer 100 ug buserelin spray every 4 hours during the day for a total daily dose 1200 ug (200 ug at bedtime) or alternatively every 6 hours for a total daily dose 800 ug. Plasma FSH and serum estradiol concentrations are measured before and after 24 hours of treatment (30). In one study the day 2 FSH level, day 3 estradiol level and the change in estradiol correlated with the number of follicles after stimulation. These authors considered a change in estradiol less than 180 pg/mL and/or FSH 9.5 IU/L to predict poor oocyte response to gonadotropins and a requirement for more gonadotropins.

Another group evaluated circulating estradiol and FSH values at 0800h before and after administration of leuprolide acetate, 1 mg sc at 1400h (31). Patients with less than a doubling of serum estradiol had an increased rate of cycle cancellation due to inadequate follicular stimulation and none became pregnant.

When is it useful?

This response may be used to to predict the response to gonadotropins and to tailor gonadotropin administration during IVF. It has not had widespread validation.

Is there a better test?

The test has not received widespread use and comparison with other tests in terms of ovarian reserve or prediction of response, and is not a standard test.

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