Hormonal Evaluation of Female Infertility and Reproductive Disorders

The two cases presented here illustrate the utility of hormone measurements in evaluating female infertility and reproductive disorders. In the discussion that follows, we outline the major tests used in evaluating female reproductive disorders, and how the results of these tests aid the clinician in formulating a diagnosis and in monitoring the efficacy of subsequent treatment.

Presentation of the Cases

Case 1: Hypothalamic Amenorrhea

Patient A was a 30-year-old white woman with a long-standing history of primary amenorrhea and infertility. By age 18 the patient had neither menstruated nor demonstrated evidence of normal breast development. At that time she was given oral contraceptives for four cycles and responded with normal vaginal bleeding. Laboratory evaluation at age 18 demonstrated a serum FSH (follicle-stimulating hormone, follitropin) value of 9 int. units/L (see Table 1 for reference intervals), a serum LH (luteinizing hormone, lutropin) value of 6 int. units/L, and a serum thyroxin concentration of 94 μg/L (normal). A laparoscopy performed later that year revealed normal but immature internal female genitalia. Several courses of clomiphene were administered, but no bleeding response was evoked. The patient again sought evaluation at age 30 because of amenorrhea, poor breast development, and infertility.

On physical examination, the patient was seen to be thin, with poorly developed breasts, infantile vulva, and a small uterus. Pubic and axillary hair were normal in appearance, and there was no evidence of galactorrhea. An epithelial smear taken from the upper third of the lateral vaginal wall revealed a markedly immature maturation index (0% superficial cells), consistent with hypoestrogenicity. Intramuscular progesterone was administered, but no withdrawal bleeding occurred. Serum FSH was 9 int. units/L. Results for serum prolactin, tomography of the sella turcica, and karyotype analysis were all normal. Challenge with gonadoliberin (gonadotropin releasing hormone) resulted in a normal increase in LH. There was a positive response (normal bleeding) to cyclic oral therapy with estrogen and progesterin.

In an attempt to become pregnant, the patient was started on pulsatile gonadoliberin infusion, with 6 μg of gonadoliberin administered subcutaneously through an indwelling line every 90 min. Initial serum estradiol values were around 40 ng/L, but increased to 250 ng/L by the 12th day of gonadoliberin infusion. Ultrasound monitoring on days 15 and 19 of therapy revealed the development and subsequent

Table 1. Hormonal Evaluation of the Adult Woman: Reference Intervals

<table>
<thead>
<tr>
<th>A. Protein hormones</th>
<th>Follicular phase</th>
<th>Ovulatory phase</th>
<th>Luteal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>8–20 int. units/L</td>
<td>&gt;25 int. units/L</td>
<td>8–20 int. units/L</td>
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<tr>
<td>FSH</td>
<td>Follicular phase</td>
<td>Ovulatory phase</td>
<td>Luteal phase</td>
</tr>
<tr>
<td></td>
<td>8–20 int. units/L</td>
<td>10–30 int. units/L</td>
<td>8–20 int. units/L</td>
</tr>
<tr>
<td>LH:FSH ratio</td>
<td>0.5–2.0</td>
<td>5–25 μg/L</td>
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<tr>
<td>Prolactin</td>
<td></td>
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B. Steroid hormones

<table>
<thead>
<tr>
<th>Estradiol</th>
<th>Follicular phase</th>
<th>Ovulatory phase</th>
<th>Luteal phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>30–150 ng/L</td>
<td>100–500 ng/L</td>
<td>50–250 ng/L</td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>Follicular phase</td>
<td>Ovulatory phase</td>
<td>Luteal phase</td>
</tr>
<tr>
<td>30–100 ng/L</td>
<td>&gt;150 ng/L</td>
<td>90–160 ng/L</td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>Follicular phase</td>
<td>Luteal phase</td>
<td></td>
</tr>
<tr>
<td>&lt;2 μg/L</td>
<td>&lt;10 μg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-Hydroxyprogesterone</td>
<td>Unstimulated</td>
<td>ACTH-stimulated</td>
<td></td>
</tr>
<tr>
<td>&lt;2.0 μg/L</td>
<td>2–3 fold increase</td>
<td></td>
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</tr>
</tbody>
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Testosterone | <0.5 μg/L |
Free testosterone | <15 ng/L |
Androstenedione | 0.5–2.8 μg/L |
DHEA | 1.4–10.1 μg/L |
DHEA-S | <3 mg/L |

*General reference ranges used in clinical practice at our institution.
<20 ng/L in patients after menopause.
Higher if patient is on ovulation induction therapy.
10-fold increase in patients with congenital adrenal hyperplasia.
rupture of a dominant ovarian follicle. After ovulation, human chorionic gonadotropin (hCG) was administered intra-muscularly for maintenance of the corpus luteum, and seven days after apparent follicle rupture the midluteal serum progesterone concentration was 22 µg/L. The intact hCG concentration in serum 18 days after ovulation was 1776 int. units/L, indicating a successfully achieved pregnancy, and 40 weeks later a healthy baby girl was delivered without incident.

Case 2: Polycystic Ovary Disease

Patient B was a 32-year-old white woman with a long-standing history of secondary amenorrhea and hirsutism. At age 22, the patient conceived and had a normal delivery. Her current complaint was secondary infertility.

Evaluation of the patient’s husband revealed severe oligo-spermia (5 x 10^6/mL) and markedly decreased sperm motility.

Values for hormones in the patient’s serum included: prolactin 7.3 µg/L, testosterone 0.95 µg/L, free testosterone 15.9 ng/L, and dehydroepiandrosterone sulfate (DHEA-S) 3.58 mg/L. A plot of basal body temperature showed no biphasic changes, and ultrasonography revealed bilateral diffusely enlarged ovaries. She had normal withdrawal bleeding in response to intramuscular progesterone.

The patient was started on clomiphene therapy, but failed to ovulate in response to the maximal dosage. When addition of dexamethasone to the clomiphene also failed to provoke an ovulatory response, the patient was treated with intramuscular human menopausal gonadotropins (purified urinary LH and FSH) for six cycles. Despite appropriate serum estradiol concentrations during this therapy and sonographic evidence of follicle rupture in some cycles, serum progesterone never exceeded 15 µg/L, even when progesterone was supplemented by means of vaginal suppositories.

Ovarian wedge resection was then performed. Just before surgery, serum androgen concentrations were again high, and values for LH and FSH were 26.9 and 8.8 int. units/L, respectively (an LH:FSH ratio of 3.1:1). One month after surgery, the concentrations of all androgens were within the normal reference interval and the serum LH and FSH were 10.9 and 15 int. units/L, respectively (an LH:FSH ratio of 0.7:1). The patient began to ovulate spontaneously, and a properly timed midluteal progesterone value two months postoperatively was 37.8 µg/L. Spontaneous ovulation continued for six months, at which point clomiphene and finally human menopausal gonadotropins were once again required to stimulate ovulation.

Two years postoperatively, the patient adopted a baby and discontinued attempts to conceive. She is currently amenorrheic once again.

Discussion

Hormonal disorders of the female reproductive system include an assortment of problems resulting from aberrant operation of the hypothalamic–pituitary–ovarian axis. These relatively common disorders often lead to infertility and are now more readily understood, diagnosed, and treated (1, 2). Measurements of peptide and steroid hormones in serum play a key role in both the investigation and the treatment of female infertility and reproductive problems (3). Appropriate testing varies widely according to the clinical presentation, physical findings, and results of other diagnostic procedures. In general, the most important hormones measured are protein hormones such as pituitary LH, FSH, prolactin, thyrotropin, placental hCG, and a variety of steroid hormones, including estrogens, progesterones, and androgens. Here we discuss female reproductive physiology, classification of female reproductive disorders, and laboratory tests for evaluating the female reproductive system.

Physiology of the Female Reproductive System

Lutropin and follitropin. The pituitary gonadotropins, LH and FSH, are members of the glycoprotein hormone family, which also includes pituitary TSH and placental hCG. All the hormones in this family are heterodimers, consisting of an α and β subunit (4–7). The α subunits of all four hormones contain the identical amino acid sequence (4–7) and are encoded by a single α subunit gene (8, 9). The β subunit, which is unique in amino acid sequence (4–7) and gene of origin (10–12), confers distinct biological and immunological specificity to the hormones (4–7, 13).

Release of LH and FSH from pituitary gonadotroph cells (14–19) is stimulated by the hypothalamic decapeptide, gonadotropin-releasing hormone, and occurs in a pulsatile fashion (13, 20–22). The regulation of gonadotropin, LH, and FSH release is maintained by a finely tuned and complex balance of positive and negative feedback mechanisms aimed primarily at hypothalamic and pituitary receptor targets. This feedback is dependent upon the concentrations of the ovarian steroid hormones in the circulation. Estradiol, the most biologically potent estrogen, plays an important role in these feedback mechanisms (13, 23, 24). LH appears to be released from the pituitary primarily under negative estradiol feedback control. As estradiol concentrations increase during follicular growth before ovulation, circulating FSH slowly but significantly decreases (see Figure 2 below). Pituitary release of LH is also closely tied to the concentration of estradiol in the circulation. As estradiol concentrations increase, the pituitary becomes sensitized for the massive burst of LH release that occurs just before ovulation. This burst is apparently triggered by the rapidly increasing estradiol secretion of the growing follicle. The declining estradiol and progesterone concentrations that occur after ovulation give rise to increased pituitary release of FSH, and the system recycles.

In females, LH stimulates synthesis by the ovarian theca cell of several androgens that are precursors for estradiol (Figure 1), causes release of the ovum from the ovarian follicle previously ripened by FSH, and promotes the formation of the corpus luteum and subsequent synthesis of progesterone. In contrast, FSH both stimulates the growth of ovarian follicles and induces conversion of androgens to estradiol by the granulosa cells (25–27). The specific effects of the various hormones, their control mechanisms, and the interrelated feedback systems function in the female to produce the menstrual cycle. In brief, the menstrual cycle can be viewed as occurring in three distinct phases (Figure 2): the follicular phase, the ovulatory phase, and the luteal phase (13, 23, 24). Each of the gonadotropins and sex steroid hormones displays characteristic concentrations and changes during each of these phases (see Figure 2 and Table 1).

Human chorionic gonadotropin. hCG, the placental hormone, is closely related structurally to the pituitary gonadotropins. It is critical for the maintenance of the corpus luteum during
early pregnancy, whereas its role in the female reproductive system before conception is as yet unknown (28). The close structural similarity between hCG and LH has allowed for the therapeutic use of exogenous hCG as an "LH analog" during ovulation induction (hCG being more readily available in purified form than LH).

Steroid hormones (estrogens, progesterone, androgens). Of more than 30 C18 estrogens that have been identified, only estradiol and its weaker relations, estriol and estrone, are frequently measured (Figure 1). Estradiol, the most biologically active and clinically important, is secreted almost exclusively by the granulosa cells of the dominant ovarian follicle. Estrone, on the other hand, is derived in large measure from the peripheral conversion of androstenedione and from the metabolism of estradiol. Estrogens of ovarian origin, in fact, account for less than half of the total estrogens in the circulation, the remainder being produced peripherally (26). Estradiol is essential for proper development of the endometrium during both the follicular and luteal phases of the menstrual cycle as well as the stimulation and maintenance of female reproductive organs and secondary sexual characteristics. Depending upon the phase of the menstrual cycle, estrogens are capable of exerting negative (follicular and luteal) as well as positive (periovulatory) feedback control of gonadotropin and gonadotropin release (25–27). In the circulation, estrogens (as well as progesterone and androgens) are largely bound to plasma proteins. However, it is the free (unbound) forms that produce the hormonal effects and feedback regulation (13).

Progesterone (C21) is produced by the corpus luteum and is present in significant amounts only during the menstrual cycle after the ovulatory phase (Figures 1 and 2). During early pregnancy, progesterone is produced by the corpus luteum and later by the placenta. In conjunction with estradiol, progesterone promotes the growth of a secretory endometrium, a prerequisite for implantation of a fertilized ovum. In addition, progesterone is thermogenic, causing the elevation in basal body temperature that occurs after ovulation (during the luteal phase).

In females, the androgens (C19) may critically act as local modulators in the selection of a dominant ovarian follicle for ovulation (29). Testosterone, dehydroepiandrosterone (DHEA), and androstenedione are the major precursors of estrogens (Figure 1). Both the ovary and the adrenal gland synthesize these androgens. Some tissue-specificity does exist, though, with androstenedione produced mainly by the ovary, the sulfated form of DHEA (DHEA-S) produced entirely by the adrenal, and testosterone produced by both organs (13). Most of the circulating testosterone and dihydrotestosterone in normal women is derived from peripheral conversion of androstenedione and, to a lesser extent, DHEA.

Prolactin. Much of the physiology of prolactin is incompletely understood. In normal women, prolactin is secreted from pituitary lactotroph cells with a marked diurnal variation and is responsive to various factors, including stress and eating, as well as tactile and non-tactile sexual arousal (30). However, no clearly isolated hypothalamic releasing factor responsible for directing its synthesis and (or) secretion has been identified. The primary sites of prolactin action appear to be the breast, the ovary, and the hypothalamus. Concentrations of the hormone increase markedly during pregnancy, from the 5–20 μg/L range found in the normal non-pregnant woman to as much as 300 μg/L at term (30). Most of this additional circulating prolactin appears to be derived from the pituitary as a result of marked hypertrophy of the lactotroph cells. In the postpartum woman, sporadic sharp increases in prolactin secretion occur as the pituitary responds to intermittent suckling, which occurs with the nursing process, and they persist even as late as six months postpartum, despite a gradual decline in the baseline prolactin concentration. Although prolactin seems to exercise a permissive effect on milk production, the hormone is not required for breastfeeding (30, 31).

Ovarian protein hormones. Recent studies have suggested that several protein hormones of ovarian origin may play important roles in the female reproductive system. For example, the peptide hormone inhibin, made by ovarian granulosa cells in the female (as well as testicular seminif-
erous tubules in the male), is now recognized as a selective feedback suppressor of pituitary FSH release (reviewed in ref. 32). Activin (an inhibin heterodimer), follistatin, follicle regulatory protein, and relaxin are other examples of recently characterized ovarian protein hormones whose functional status and clinical relevance remain largely unclear (33).

Utility of Laboratory Tests for Evaluating the Female Reproductive System

Classification of Disorders

A. Hypoestrogen disorders. The presence of low estrogen concentrations in serum is, by definition, diagnostic of hypogonadism. Estrogen deficiency can be demonstrated by directly measuring estradiol in the patient's serum but is more typically assessed physiologically by a menstrual response to a course of exogenous progesterone (2, 34). Vaginal bleeding after administration of progesterone suggests the presence of estrogen-stimulated endometrium, whereas failure to bleed implies that little endometrium is present and that endogenous estrogen secretion is depressed (34, 35).

Hypogonadism occurs in two forms: that with co-existing increased pituitary gonadotropin concentrations (hypergonadotrophic hypogonadism) and that with co-existing decreased pituitary gonadotropin concentrations (hypogonadotrophic hypogonadism) (Table 2). After establishing that there is a deficiency of serum estrogen, it is always important, Therefore, to determine whether the hypoestrogenic state is accompanied by an increase or decrease of pituitary gonadotropin concentrations.

Hypergonadotrophic hypogonadism. The presence of elevated LH (>25 int. units/L) and particularly FSH (>40 int. units/L) concentrations with a decreased estradiol (<20 ng/L) concentration is diagnostic of hypergonadotrophic hypogonadism (gonadal failure) (36). The origin of hypergonadotrophic hypogonadism is always the failure of ovarian follicles to produce estradiol upon gonadotropin stimulation. In the absence of estradiol negative feedback, the pituitary produces greater amounts of FSH in an attempt to produce an ovarian estradiol response. Thus, in these disorders, FSH concentrations are more consistently supranormal than are those of LH, since the absence of negative feedback by ovarian estradiol has a more pronounced effect on FSH. In fact, FSH values >40 int. units/L are typically associated with ovarian failure. Four major areas of hypergonadotrophic hypogonadism may be identified (Table 2).

Menopause, by far the most common cause of static gonadotropin elevation (29), affects women in the 40–55 age group. The appearance of characteristic symptoms (loss of regular menses, hot flushes, skin dryness, and decreased vaginal lubrication) and physical findings (loss of vaginal rugation and decreased uterine size) usually make the diagnosis an easy one for the clinician. Failure to respond to a progesterone challenge and a loss of estrogen effect on vaginal maturation index are also simple and inexpensive options for making the diagnosis of menopause. Serum gonadotropin concentrations may be misleading during the perimenopause, because most women will undergo only intermittent periods of increase before the final development of amenorrhea (37).

Premature ovarian failure, one of the variations of normal menopause, is defined as the disappearance of normal female menstrual function before age 40. Possible etiologies include infections, autoimmune phenomena, cytotoxic drug or radiation damage, and altered sex chromosome content (38). Clinical and laboratory diagnoses are identical to those mentioned above for normal menopause. Phenotypically female gonadal dysgenesis, an uncommon but extremely important etiology for hypergonadotrophic hypogonadism, includes X0 gonadal dysgenesis (Turner's syndrome), XY gonadal dysgenesis (Swyer's syndrome), XX gonadal dysgenesis, and a variety of mosaic patterns involving duplications and absence of the X or Y chromosomes (39–41). Typically, these women will have streak gonads without germ cells and normal, but infantile, internal and external female genitalia. The resistant-ovary syndrome (Savage syndrome), another of the menopausal-like disorders, is diagnosed by the failure of an ovarian estradiol response to markedly increased pituitary gonadotropin concentrations in the presence of otherwise normal ovarian follicles (42–44). Unlike other causes of hypergonadotrophic amenorrhea, many women with this syndrome will spontaneously resume ovarian function and ovulation after variable periods of hypoestrogenicity. Savage syndrome is rare. In fact, as a general rule, more than 95% of women under age 40 with extended hypoestrogenicity and serum FSH values >40 int. units/L do not have this disorder and will never resume normal ovarian function (45).

Hypergonadotrophic hypogonadism. The co-existence of decreased LH (<10 int. units/L), FSH (<10 int. units/L), and

| Table 2. Characteristic Laboratory Findings: Hypoestrogenic Disorders |
|-------------|-------------|----------------|-----------------------------|
| Estradiol   | FSH and LH  | Additional testing | Relative           |
| Menopause   | ↑           | ↑               | Karyotype                  |
| Gonadal dysgenesis | ↑            | ↑               | Uncommon                   |
| Premature ovarian failure | ↑            | ↑               | Rarer                      |
| Ovarian resistance syndrome | ↑            | ↑               | Very rare                  |
| Hyperprolactinemia | ↓           | Ni or ↓         | TSH, prolactin             |
| Weight-loss amenorrhea | ↓          | ↓               | Common                     |
| Exercise amenorrhea | ↑           | ↓               | Common                     |
| Anorexia nervosa | ↓           | ↓               | Uncommon                   |
| Kaliman's syndrome | ↓          | ↓               | Anosmia testing            |
| Pituitary failure (Sheehan's syndrome, Ahumada-del Castillo syndrome) | ↓          | ↓               | Somatotropin, TSH, ACTH    |

Ni, normal.

CLINICAL CHEMISTRY, Vol. 35, No. 4, 1989 623
estradiol (<20 ng/L) values is diagnostic of hypogonadotropic hypogonadism (central gonadal failure) (36). With these disorders, a pituitary or hypothalamic defect is responsible for the failure of gonadal steroid production (Table 3). The failure to secrete (or occasionally to recognize) gonadotropins is responsible for Kallman’s syndrome, a familial disorder of hypogonadotropic hypogonadism with anosmia (see Case 1) (46). Kallman’s syndrome was first described in males, where it results in sterility and eunuchoid features. Other common causes of hypogonadotropic hypogonadism include anorexia nervosa, excessive weight loss or exercise, hyperprolactinemia, and pituitary failure (47).

B. Hirsutism/virilization disorders. Androgen excess is a fairly common disorder of the premenopausal woman (2). Its most severe manifestation, virilization, is rare and almost always associated with androgen-secreting tumors or exogenous hormone administration (48). Excessive growth of facial or midline hair in the female (hirsutism) is seen far more frequently. The laboratory hallmarks in the diagnosis of hirsutism are the values of serum adrenal and gonadal androgens. The changes in these tests and some other useful additional laboratory assays such as serum prolactin, 3α,17β-androstanediol glucuronide, adrenocorticotrophic hormone (ACTH)-stimulated 17-hydroxyprogesterone, and various pituitary glycoprotein hormones are shown in Table 3. Serum testosterone concentrations will be increased in all but those women with idiopathic hirsutism. Evaluation of DHEA-S concentrations is critical, as disproportionate elevations of this adrenal-specific androgen suggest the diagnosis of adrenal hyperplasia or tumor (48). Concurrent increases of total and free testosterone concentrations suggest an ovarian etiology for hirsutism, but androstenedione and DHEA are also measured in some centers as an adjunctive means of evaluating hyperandrogenism.

Hirsutism may have a variety of etiologies, including increased cellular androgen sensitivity, ovarian or adrenal androgen-secreting tumors, late-onset congenital adrenal hyperplasia, and iatrogenic administration (49). The most common treatable cause of hirsutism, however, remains the polycystic ovary disease.

Polycystic ovarian disease is characterized by the symptom triad of hirsutism, oligoovulation, and infertility. Bi-manual or sonographic evaluation usually demonstrates characteristic bilateral ovarian enlargement (60). Free and total testosterone concentrations are usually disproportionately increased when compared with DHEA-S. The diagnosis of polycystic ovarian disease is supported by an LH:FSH ratio of greater than 3:1 (see Case 2) (48, 51). Concentrations of serum testosterone exceeding 2 μg/L are typically associated with the presence of an androgen-secreting ovarian tumor, as are free testosterone values >40 ng/L. DHEA-S values >8 mg/L have been associated with androgen-producing adrenal tumors (48). It is also important to remember the association of mild hirsutism with the endocrine disorders of Cushing’s disease, acromegaly, and especially hyperthyroidism (32).

C. Ovulation disorders. Ovulation disorders are relatively common problems, occurring in as many as 10% of premenopausal women (24). Although the great majority of cases have no apparent etiology, causes of anovulation that can be diagnosed vary widely. Hyperprolactinemia may account for up to 10% of cases. Thyroid, renal, and collagen vascular diseases, as well as diabetes, are uncommon but important etiologies. Many systemic medications can produce either temporary or extended bouts of anovulation. Important laboratory evaluation of the anovulatory woman might, therefore, include serum prolactin, TSH, thyroxin, and hCG values, as well as preprandial blood glucose and screening tests for collagen vascular disease (anti-nuclear antibodies), as deemed appropriate to history and physical examination findings. The differential diagnosis of abnormal uterine bleeding should also include pregnancy and its disorders, iatrogenic hormone administration, mechanical problems (intrauterine devices, endometrial polyps, and myometrial leiomyomas), and cancers of the reproductive tract. Great efforts should be made to rule out these disorders before making the diagnosis of anovulation.

The simplest technique for the detection of ovulation is the measurement of basal body temperature. The production of progesterone, a thermogenic steroid, in the luteal phase is heralded by an increase of 0.3–1.0 °F in the early morning basal body temperature (Figure 2). The classic "biphasic" ovulatory pattern reflects the fact that the basal body temperature is relatively low during the follicular phase, shifts abruptly within 24 hours of ovulation, and generally remains high for the 12–14 days of the luteal phase.

The critical laboratory test for evaluating ovulation is serum progesterone measurement. Normal women experience an increase in serum progesterone concentrations, beginning just after ovulation and peaking approximately five to nine days later (midluteal). If pregnancy does not occur, progesterone values then typically drop two to three days before menstruation, eventually approaching zero during the bleeding period itself. Because precise timing of ovulation may be difficult to ascertain and because the luteal phase is nearly always about 14 days in length, proper midluteal timing of serum progesterone sampling is best verified by retrospectively establishing that the test was performed four to eight days before the onset of menses. Radwanska et al. (53) established normal midluteal progesterone values for ovulatory women; >10 μg/L reflecting normal ovulation, whereas <10 μg/L suggests anovulation, inadequate luteal phase progesterone production, or, most

<table>
<thead>
<tr>
<th>Table 3. Characteristic Laboratory Findings: Hirsutism/Virilization Disorders</th>
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<tr>
<td>Polycystic ovarian disease</td>
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<tr>
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<td>Congenital adrenal hyperplasia</td>
</tr>
<tr>
<td>Hormonal condition:</td>
</tr>
<tr>
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<tr>
<td>Hyperthyroidism</td>
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<tr>
<td>Acromegaly</td>
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<td>Ovarian tumor</td>
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624 CLINICAL CHEMISTRY, Vol. 35, No. 4, 1989
commonly, inappropriate timing of the test. Progesterone values as high as 15–20 μg/L often occur in women undergoing ovulation induction with clomiphene or human menopausal gonadotropins, because serum midluteal progesterone is directly related to the number of corpora lutea present during the luteal phase (54). Determinations of salivary or urinary progesterone concentrations, although offering more convenience to the patient (2, 55–57), are generally more cumbersome and less accurate than determinations in serum. These problems have made their use uncommon in the United States. Ovulation (and ovum release) may also be accurately determined by using modern transvaginal ultrasonography (58). Such testing, often performed in conjunction with appropriately timed midluteal progesterone assessment, currently offers the most definitive evidence of normal ovulation.

Recently, urinary LH has been measured to assess ovulation (59). These tests, performed by the patient, rely upon the abrupt appearance of LH in the urine shortly after the physiological LH surge. The surge of LH in the normal menstrual cycle occurs 24–36 h before ovulation (60), so most commercially available procedures will reveal increases in urinary LH at or near the day of ovulation. The tests are relatively easy to perform, are effective in determining ovulation in about 70% of women, and, at least in theory, can be used for timing intercourse, artificial insemination, and oocyte recovery (59). These tests, however, cannot be readily utilized for establishing the presence or evaluating the etiology of anovulation.

D. Early pregnancy evaluation. The laboratory evaluation of early pregnancy is used to verify the presence of normal pregnancy as well as its major complications (Table 4). The clinical diagnosis and management of normal early pregnancy and early pregnancy disorders are guided by three types of evaluations: serum hCG, serum progesterone, and ultrasonography. In normal pregnancy, serum hCG may first be detected as early as a few days before a missed menstrual period and logarithmically increases to a peak around the eighth or ninth postmenstrual week (61). Progesterone concentrations will usually remain above 15 μg/L, generally reaching the 30 μg/L range at about the time the placenta begins to add significant progesterone production to that already achieved by the ovaries (62), usually near the end of the first trimester of pregnancy. Obstetrical ultrasonound, most accurately performed vaginally by use of a high-frequency transducer system, can provide critical information regarding pregnancy location and viability as early as one week after a missed menstrual period (63, 64). The crucial ultrasonographic finding during normal early pregnancy is the presence of a viable first-trimester intrauterine fetus. This is generally detected between five and seven weeks of gestation and at hCG concentrations between 1000 and 5000 int. units/L (65).

The blighted intrauterine pregnancy, which inevitably results in a spontaneous pregnancy loss, may be associated with serum hCG values similar to those seen in normal pregnancy and either normal or decreased progesterone concentrations (Table 4). Typically, blighted intrauterine pregnancies are without evidence of a fetus or fetal motion by vaginal ultrasound. Ectopic pregnancies are associated with progesterone values that are typically <15 μg/L, the absence of a true intrauterine gestational sac by ultrasonography, and serum hCG values that tend to be low and fail to demonstrate an appropriate increase over time. Romero and coworkers (66, 67) proposed that the change in concentration of two serum hCG samples drawn 48 h apart in a viable first-trimester intrauterine pregnancy should be greater than 66%. hCG concentrations that increase at this rate are doubling approximately every 2.2 days. The term “doubling time” has come into common usage to describe this pattern of serum hCG increase at two-day intervals. The rare pregnancies that involve development of hydatidiform moles are characteristically detected by either unusually high serum hCG values (68) or grossly abnormal ultrasound studies. Additional details for using hCG as a marker of pregnancy and malignancies have been described (68–72).

E. Hyperprolactinemic states. The primary pathophysiological importance of prolactin resides in the clinical manifestations of increased serum concentrations of the hormone (hyperprolactinemia) (73). Evaluation of serum prolactin is important for establishing a diagnosis not only for those patients with galactorrhea but also for women with a variety of other reproductive disorders. Hyperprolactinemia may be involved in up to 20% of cases of secondary amenorrhea (34) and also plays a significant role in the etiology of oligomenorrhea and infertility. The occurrence of hyperprolactinemia secondary to a pituitary tumor can be associated with visual changes and headaches, but these symptoms generally result from tumor expansion rather than primary effects of the hormone. Such symptoms of hyperprolactinemia and pituitary growth are usually insidious in nature and have a gradual onset (74, 75).

Laboratory Tests

A. Lutropin and follitropin. Immunoassays for LH and FSH have essentially replaced the more time-consuming and expensive bioassays. In general, the immunoassays utilize antisera specific for the unique β subunits, thereby allowing discrimination of these structurally related glycoproteins with <1% cross-reactivity (13, 76). Whereas most antisera to FSH display virtually no cross-reactivity with hCG or TSH, antisera to LH often demonstrate significant cross-reactivity with hCG and, with some antisera, TSH. Thus, LH measurements are generally invalid in pregnant individuals, in patients with hCG-secreting tumors, and in some hypothyroid subjects. The latter may become less of a problem with the advent of immunometric assays using highly specific monoclonal antibodies (76, 77). Immunoassay reagents for measuring LH and FSH are now widely available (>20 commercially available assay systems), and the techniques for their use are reasonably sensitive, precise, accurate, and easy to perform on a routine basis (for review see refs. 78 and 79). These assays typically achieve sensitivities on the order of 1.0 and 0.5 int. unit/L for LH and FSH, respectively.

Plasma or serum can be used to measure LH and FSH

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| Table 4. Characteristic Laboratory Findings in Early Pregnancy Evaluation |
|----------------------------------|--------|---------|
| Normal intrauterine pregnancy   | N<sup>1</sup> | NI<sup>1</sup> |
| Blighted intrauterine pregnancy| N<sup>1</sup> or ↓ | ↓ |
| Ectopic pregnancy              | ↓      | Variable |
| Hydatidiform mole              | ↑      | Variable |

<sup>1</sup> Gestation <8 weeks from the last menstrual period.

<sup>2</sup> Normal values depend upon gestational age, assay type, and reference preparation standard.
concentrations. No specific patient preparation is required, and no marked circadian variations seem to occur with the pituitary gonadotropins. However, the release of gonadolib-erin occurs in episodic bursts every 60–90 min, resulting in discrete pulses in the concentrations of serum LH and, to a lesser extent, FSH. Although several studies (20–22, 78, 80) examining the pulsatile release of LH and FSH have suggested that multiple, pooled serum samples be used for gonadotropin measurements, most clinicians use a single, unpooled sample for testing. Reference intervals for LH and FSH, which vary with the phases of the menstrual cycle (follicular, ovulatory, luteal), are well established (see Table 1) (13).

Serum gonadotropins typically are measured under steady-state conditions. Dynamic testing, which involves measuring the response of LH and (or) FSH concentrations to specific stimuli (such as gonadoliberin), is reserved for a few well-defined situations (2, 34, 81).

B. Human chorionic gonadotropin. Serum hCG can be measured using antisera specific for the β subunit (69) or, more recently, utilizing antibodies to both the α and β subunits, making the assay specific for intact α-β hCG dimers (82). The sensitivity of these newer immunometric assays is typically 1 int. unit/L, allowing the detection of pregnancy as early as seven days after conception (28).

C. Steroid hormones. Determinations of steroid hormone concentrations by sensitive immunoassays (83), which assess both free and protein-bound hormone, have largely replaced the chemical, chromatographic, and competitive binding techniques. Although serum concentrations of the steroid hormones are the standard for clinical evaluation, these compounds may also be measured in plasma, urine, and, more recently, saliva (84). As with the gonadotropins, reference ranges for the various sex steroid hormones are well established and are highly specific for the patient’s age and the phase of her menstrual cycle (13). Commonly measured sex steroid hormones include estradiol, progesterone, 17-hydroxyprogesterone, free and total testosterone, DHEA-S, androstenedione, and 3a,17β-androstanediol glucuronide.

D. Prolactin. Prolactin concentrations are typically measured in the clinical laboratory by immunoassay. For the most nearly accurate measurements, serum samples should be obtained during the morning in the absence of a high protein meal, with the patient receiving only chronic medications, and after a quiet period to eliminate any undue stress (73–75). Two common mistakes are to obtain serum for prolactin measurement after a rigorous breast examination or after a large, protein-rich meal; such a value will not reflect the patient’s normal baseline prolactin concentration, generally producing values in the mildly increased range (73–75). High values for serum prolactin should be verified under optimal conditions before proceeding with extensive radiological and therapeutic measures.

Summary of Cases

The first case represents a young woman with primary infertility, who presents with the typical history, physical features, and laboratory findings (decreased serum LH, FSH, and estradiol concentrations) of hypogonadotropic hypogonadism (Table 2). In this case, the hypogonadogenicity and resulting amenorrhea were secondary to inadequate hypothalamic gonadoliberin secretion. This relatively common cause of primary amenorrhea is often accompanied by anaemia (absence of the sense of smell) and has been given the name Kallman’s syndrome. In females, the hypothalamic defect can be partly corrected by replacing the gonadal steroids (estrogens and progesterone) that are not being produced owing to a lack of gonadotropin stimulation. This simple therapy results in normal development of female secondary sexual characteristics and monthly menstruation. If pregnancy is desired, follicular development and ovulation can be achieved by pulsatile infusion of gonadoliberin or intramuscular administration of human menopausal gonadotropins. The correct identification of the cause of primary amenorrhea as uterine, ovarian, pituitary, hypothalamic, or systemic in origin is critical.

The second case illustrates a typical patient with secondary amenorrhea and infertility due to polycystic ovary disease. Before performing diagnostic and therapeutic studies on these women, it is important to rule out (via testing for urinary or serum hCG) the most common cause of loss of menses: pregnancy. Polycystic ovary disease is the second most common cause of secondary amenorrhea during the reproductive years. The hallmark laboratory findings in this disorder include a nearly 10-fold increase in ovarian and adrenal androgens and an increased LH:FSH ratio (Table 3). Additional support for the diagnosis can be provided by sonographic or laparoscopic evidence of diffuse ovarian enlargement and the presence of multiple immature ovarian follicles. Physical examination may not be helpful, because a significant proportion of these women fail to demonstrate the typical obese, hirsute appearance described by Stein and Leventhal (85). Unlike this patient, most women with polycystic ovary disease will ovulate in response to modest doses of clomiphene. The role of polycystic ovary disease in this patient’s infertility is more difficult to define, because her husband demonstrated oligospermia and sperm hypomotility. If pregnancy is not desired in a patient with polycystic ovary disease, the hirsutism may be controlled with either oral contraceptives or spironolactone therapy. For those patients desiring pregnancy, surgical removal of a portion of an ovary (ovarian wedge resection) is often a successful, temporary endocrine therapy, but has at least a 25% incidence of pelvic wedge adherence and should be reserved for only the most recalcitrant cases of anovulatory infertility due to polycystic ovary disease (86–88).

Summary

Performance of the male and female reproductive systems reflects the orderly operation of the hypothalamic–pituitary–gonadal axis. Aberrant operation of this axis can result in many different reproductive disorders, including various forms of infertility. Proper evaluation of these disorders involves a multifaceted diagnostic approach, which includes a critical contribution from the clinical laboratory. This adjunctive testing, involving the measurements of peptide and sex-steroid hormone concentrations, allows the clinician to biochemically “dissect” the hypothalamic–pituitary–gonadal axis and ascertain the presence as well as location of the specific defect. In practice, the specific tests utilized during the evaluation of a patient depend upon the underlying disorder. Typically, in evaluating the reproductive disorders discussed in this review, a primary battery of tests is obtained that reflects the initial clinical presentation and physical examination. The results of these initial studies then dictate any secondary testing required to complete the evaluation. Such an approach, in use at our institution, is provided in Table 5.

Although this discussion has concentrated on the labora-
Table 5. Temporal Testing Strategy for Evaluating Female Reproductive Disorders*

<table>
<thead>
<tr>
<th>A. Hypoestrogenic disorders</th>
<th>Primary tests:</th>
<th>Secondary tests:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone challenge, vaginal maturation index, serum FSH</td>
<td>Serum prolactin, serum LH:FSH ratio, serum estradiol, radiographic studies of pituitary, karyotype, serum hCG</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Hirsutism/ovarian disorders</th>
<th>Primary tests:</th>
<th>Secondary tests:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum testosterone, serum free testosterone, serum DHEA-S</td>
<td>Serum 3α,17β-androstenediol glucuronide, serum 17-hydroxyprogesterone (ACTH-stimulated), serum TSH, serum cortisol (morning), serum somatotropin; serum LH:FSH ratio</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Ovulation disorders</th>
<th>Primary tests:</th>
<th>Secondary tests:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum hCG, basal body temperature plot, serum progesterone, serum prolactin</td>
<td>Ultrasonography, urinary LH, serum TSH</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. Early pregnancy evaluation</th>
<th>Primary tests:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum hCG, serum progesterone, ultrasonography</td>
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<td></td>
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</tbody>
</table>

*General sequence of testing performed at our institution during evaluation of the major classes of female reproductive disorders discussed in this review.

tory assessment of the female reproductive system, it is important to remember the special case of infertility, where couples, in general, are evaluated together by the clinician. The cause of infertility can reside with the female, the male, or, in the cases of immunological "incompatibilities," a combination of the male and the female. As such, rigorous schemes for evaluating male reproductive disorders (1, 3, 89–94) and immunological incompatibilities (95–98) have been developed, and the information derived from such testing represents a critical contribution to establishing the etiology of a couple's infertility.

Although the laboratory assessment of peptide and sex steroid hormone concentrations clearly plays a pivotal role in the evaluation of reproductive disorders, these diagnostic tests probably will continue to change and improve in the years to come. Such changes will probably occur as the finer details of the operation of the hypothalamic–pituitary–gonadal axis become known. With this improved knowledge, we should have the capacity to design assays that will allow more clinically refined and biochemically precise means of diagnosing and treating specific reproductive disorders.

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