The incidence of infertility has been increasing over the last several decades. Often, the clinical presentation suggests that the cause of infertility may be endocrine in nature.

For these cases, the clinical laboratory plays an important role by performing testing for various reproductive hormones to elucidate the cause of the infertility and confirm the diagnosis.

Because of the vital role of the laboratory in infertility assessment, it is important that its assays produce accurate and reliable results.

Infertility, the inability to conceive after 1 year of unprotected intercourse, affects about 10% to 20% of American couples. Assessment of infertility involves evaluating each partner to elucidate the cause. Thus, the causes of infertility are grouped by sex and include: unknown etiology (10%),2,4,14 all diseases, disorders, and problems associated with endocrines.

The hypothalamus, a small structure located deep in the brain, synthesizes gonadotropin-releasing hormone (GnRH), a small decapptide. Synthesis and release of this hormone is regulated by central nervous system stimuli (neurotransmitters, neuromodulators, and stress). Sex steroids are synthesized and released both through positive and negative feedback loops. Pituitary hormones, such as luteinizing hormone (LH) and follicle stimulating hormone (FSH), are synthesized and released through short negative feedback loops. Testosterone in males and estrogen in females provide negative feedback control of GnRH, while estrogen regulates via positive feedback in the late follicular phase in menstruating females.

Gonadotropin-releasing hormone is released from the hypothalamus in a pulsatile fashion to stimulate the gonadotrope cells of the anterior pituitary, a small gland located in the base of the skull in the sella turcica bone cavity. The stimulated gonadotropes synthesize and pulsatilely release the pituitary hormones, LH and FSH, differentially depending on the frequency of the GnRH pulses. In females, both LH and FSH secretion are controlled by negative feedback by estrogen; LH secretion is also controlled by positive feedback by rising estrogen levels just prior to ovulation; and FSH secretion is inhibited by the peptide inhibin, which is produced by the ovarian granulosa cells, especially in older women. In males, both LH and FSH secretions by the pituitary are controlled by negative feedback from testosterone; FSH secretion is also selectively inhibited by inhibin produced by the seminiferous tubules.

The LH and FSH of the pituitary stimulate the ovaries in the female to produce various sex steroids, including estrogens, androgens, and progestins. Estradiol, the most potent natural estrogen, is responsible for the pubertal development of the female secondary sex characteristics.
### Male Factors of Infertility

**Hypothalamic/pituitary disorders**
- Panhypopituitarism
- Hypothalamic syndromes
- Structural defects
- Prader-Willi syndrome
- Laurence-Moon-Biedl syndrome
- Isolated LH/FSH deficiency
- Hyperprolactinemia
- Malnutrition and anorexia nervosa
- Drug-induced suppression of LH

**Gonadal abnormalities**
- Acquired (irradiation, orchitis, castration)
- Chromosomal
- Klinefelter's Syndrome
- True hermaphroditism
- Defective androgen synthesis
  - 20-α-hydroxylase deficiency
  - 17,20-hydroxylase deficiency
  - 3-β-hydroxysteroid dehydrogenase deficiency
- 17-α-hydroxylase deficiency
- 17-β-hydroxysteroid dehydrogenase deficiency
- 5-α-reductase deficiency
- Testicular agenesis
- Selective seminiferous tubular disease
- Miscellaneous
  - Noonan's syndrome
  - Streak gonads
  - Myotonic dystrophica
  - Cystic fibrosis

**Defects in androgen action (pseudohermaphroditism)**
- Complete androgen insensitivity (testicular feminization)
- Incomplete androgen sensitivity
- Testosterone receptor defect
- Testosterone postreceptor defect
- 5-α-reductase deficiency

**Other causes**
- Obstruction or congenital absence of the vas deferens
- Varicocele
- Retrograde ejaculation
- Infections (chlamydia, syphilis)
- Genetic disorders, including Klinefelter's syndrome
- Hypothyroidism

### Female Factors of Infertility

**Anatomic abnormalities**
- Vaginal disorders
  - Vaginal aplasia
  - Imperforate hymen
  - Congenital vaginal atresia
- Uterine disorders
  - Congenital absence of the uterus
  - Endometriosis
  - Pregnancy
  - Post-traumatic uterine synchie
  - Progestational agents
- Ovarian disorders
  - XO gonadal dysgenesis
  - XX gonadal dysgenesis
  - XY gonadal dysgenesis
  - Congenital absence of the gonad
  - Testicular feminization syndrome
  - 17-hydroxylase deficiency of the ovaries and adrenals
  - Autoimmune oophoritis
  - Resistant ovary syndrome
  - Polycystic ovary syndrome
  - Ovarian tumors
  - Precocious menopause
- Adrenal disorders
  - Congenital adrenal hyperplasia
  - Cushing's syndrome
  - Virilizing adrenal tumors
  - Adrenocorticotoid insufficiency
- Thyroid disorders
  - Hypothyroidism
  - Hyperthyroidism
- Pituitary-hypothalamic disorders
  - Hypopituitarism
  - Constitutional delay in the onset of menses
  - Nutritional disorders
  - Hyperprolactinemia
  - Tumors and infiltrative diseases
  - Central nervous system disorders

In the adult female, FSH stimulates the growth and development of the ovarian follicles which produce and secrete estradiol during the follicular phase of the menstrual cycle. These estrogens, in turn, stimulate the proliferation of the uterine endometrium, increase the thickness of the vaginal epithelium, increase the vascularity of the cervix, increase cervical mucus elasticity, and dilute the cervical os. Luteinizing hormone is also responsible for stimulating the ovarian synthesis of androstenedione and testosterone, which are subsequently converted to estrogens. In the female, testosterone is also produced by the adrenals and through the peripheral metabolism of weak adrenal androgens, such as androstenedione, in adipose tissue.

In the adult male, LH stimulates the interstitial Leydig cells of the testes to synthesize and secrete the testosterone necessary for sperm production. Follicle stimulating hormone promotes spermatogenesis in the seminiferous tubules by stimulating the Sertoli cells to produce androgen-binding protein (ABP), which is required to maintain locally high concentrations of testosterone necessary for effective spermatogenesis. In males, testosterone is responsible for the masculine differentiation of the fetal genital tract, the development and maintenance of male secondary sex characteristics, and spermatogenesis. The testes also produce estradiol in small amounts.

### Reproductive Endocrine Disorder Classifications

Reproductive endocrine disorders are due to either hyperfunction or hypofunction of an endocrine gland. Classification of this hyper- or hypofunction is primary, secondary, or tertiary, depending on the site of the defect—primary for the target organ, secondary for the pituitary, and tertiary for the hypothalamus. When considering any hormonal disturbance, the patient presentation and laboratory test results help delineate the disorder as either hypo- or hyperfunction. F1 depicts the classification of defects based on target and tropic hormone levels. A summary of the reproductive endocrine disorders is given in T3.
defects, gonadal defects, or defective androgen action.

Males with a low serum testosterone and elevated LH and FSH are classified as having hypergonadotropic hypogonadism and have a primary testicular disorder. Gonadal failure may be acquired (mumps orchitis, radiation, castration), genetic [Klinefelter’s syndrome (47XXY – phenotypically male, though eunuchoid, with small testes and with azoospermia)], related to a deficiency of an androgen synthetic enzyme, or caused by the male climacteric (Leydig cell failure in older men).

Hypogonadotropic hypogonadism, characterized by decreased serum concentrations of LH, FSH, and testosterone, is rooted in secondary functional abnormalities at the level of either the hypothalamus or pituitary. Endocrine causes include panhypopituitarism, hypopituitarism, hypothalamic syndromes, and hyperprolactinemia. Causes are related to central disorders including anorexia nervosa, malnutrition, stress, intense physical training, weight loss, and certain medications. Two genetic syndromes, Prader-Willi (characterized by sexual infantilism) and Kallman (hypothalamic deficiency

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**Reproductive Endocrine Disorders**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Classification</th>
<th>Results</th>
<th>Major Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Hypogonadotropic hypergonadism (Primary ovarian hyperfunction)</td>
<td>↓ FSH and LH ↑ Estradiol</td>
<td>Estrogen-secreting tumors</td>
</tr>
<tr>
<td></td>
<td>Hypergonadotropic hypergonadism (Primary ovarian hyperfunction)</td>
<td>↑ FSH and LH ↓ Estradiol</td>
<td>Turner’s Syndrome (45XO) Menopause Polycystic ovary disease Testicular feminization</td>
</tr>
<tr>
<td></td>
<td>Hypogonadotropic hypergonadism (Secondary pituitary/hypothalamic hyperfunction)</td>
<td>↓ FSH and LH ↓ Estradiol</td>
<td>Hypopituitarism Hypothalamic disorders Hypothyroidism Hyperprolactinoma Hyperandrogenemia Pregnancy Central disorders (anorexia, stress, intense physical training, weight loss, malnutrition) Certain medications</td>
</tr>
<tr>
<td>Male</td>
<td>Hypergonadotropic hypergonadism (Primary testicular hyperfunction)</td>
<td>↑ FSH and LH ↓ Testosterone</td>
<td>Mumps orchitis Radiation Enzyme deficiency Male climacteric Klinefelter’s syndrome (47XXY) Complete androgen insensitivity (testicular feminization) Incomplete androgen insensitivity Certain adrenogenital disorders</td>
</tr>
<tr>
<td></td>
<td>Hypogonadotropic hypergonadism (Secondary pituitary/hypothalamic hyperfunction)</td>
<td>↓ FSH and LH ↓ Testosterone</td>
<td>Panhypopituitarism Hypothalamic disorders Prader-Willi syndrome Kallman’s syndrome Hyperprolactinemia Hypopituitarism Central disorders (anorexia, stress, intense physical training, weight loss, malnutrition) Certain medications</td>
</tr>
<tr>
<td>Male/Female</td>
<td>Hypergonadotropic hypergonadism (Secondary pituitary/hypothalamic hyperfunction)</td>
<td>↑ FSH and LH ↑ Estradiol</td>
<td>Precocious puberty</td>
</tr>
</tbody>
</table>

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of GnRH), are classified under hypogonadotropic hypogonadism.

In males with normal serum testosterone levels but with evidence of hypoandrogenization, end organ resistance to sex hormones is suspected (complete or incomplete androgen insensitivity).

**Female Reproductive Endocrine Disorders**

Normal menstrual periods in an adult female imply normal levels of FSH, LH, and estradiol and thus no reproductive endocrinology disorder. Female reproductive disorders are due to either reproductive hormone hypofunction or hyperfunction. The clinical presentation of female hypogonadism depends also on the age of onset and the degree of estrogen deficiency. Variations in presentation include ambiguous genitalia, delayed puberty, postpubertal gonadal failure, and infertility.

Female hypergonadotropic hypogonadism, caused by primary ovarian hypofunction, is characterized by increased levels of serum LH and FSH and decreased levels of serum estradiol. Causes of primary female hypergonadotropic hypogonadism include Turner’s syndrome (45 XO – phenotypically female with infantile sexual development and ovaries containing no follicles), menopause, testicular feminization (androgiinsensitivity - phenotypically female, genotypically male), and polycystic ovary disease.

With female hypergonadotropic hypogonadism, the level of the defect is either secondary (at the level of the pituitary) or tertiary (at the level of the hypothalamus). Typically, estradiol, progesterone, LH, and FSH are all decreased. Causes include hypopituitarism, hypothalamic disorders, hypothryoidism, hyperprolactinemia, hyperandrogenemia, pregnancy, central disorders (anorexia nervosa, stress, intense physical training, weight loss, and malnutrition) and certain medications, such as GnRH analogues.

Female hypogonadotropic hypogonadism, characterized by decreased levels of LH and FSH but increased estradiol concentrations, has estrogen-secreting tumors as its root cause. The high levels of estradiol effectively shut down the hypothalamic-pituitary axis through negative feedback and thus prevent the development of normal ovulatory menstrual cycles.

The major causes of female secondary hypergonadism, hypergonadotropic hypergonadism, are precocious puberty and the premature maturation of the CNS. Estradiol, LH, and FSH are all inappropriately increased for the age of the child.

**Clinical Assessment of Infertility**

The diagnostic assessment for infertility is based on the clinical presentation of each sex partner as determined from physical history. The major clinical presentations associated with infertility by sex are presented below.

**Male Infertility Clinical Presentations**

*Impotency*: In men, the initial presentation of hypogonadism may be impotency and/or infertility. For those 15% to 20% of impotency cases that are hormonal in nature, initial assessment includes the analysis of serum total or free testosterone. The additional measurement of serum prolactin identifies those men with impotency secondary to hyperprolactinemia.

*Gynecomastia*: Gynecomastia is the increase in the non-fatty tissue of the male breast. If the estrogen:androgiin ratio increases, the breast tissue enlarges. Alterations in the concentration of sex hormone binding globulin (SHBG) can also be a source of gynecomastia. Causes of gynecomastia include puberty (50% to 70% of normal boys), cirrhosis of the liver, chronic renal failure, medications (estrogens and digitalis), hyperthyroidism, rapid weight gain, primary testicular failure, hypogonadotropic hypogonadism, and an estrogen- or hCG-secreting tumor.

Evaluation of gynecomastia involves testing serum levels of LH, FSH, testosterone, prolactin, and hCG. Sometimes, particularly when gynecomastia is accompanied by testicular atrophy (suggesting Kleininfeller’s syndrome), chromosome analysis may be required.

**Female Infertility Clinical Presentations**

*Amenorrhea*: Women with ovulatory dysfunction do not have regular menstrual cycles occurring every 26 to 35 days and the associated premenstrual symptoms, including breast tenderness, lower abdominal bloating, and moodiness. Amenorrhea, the absence of menstruation, is abnormal unless associated with prepuberty, pregnancy, lactation, or menopause. Pathological causes of amenorrhea include anatomic abnormalities, genetic defects, ovarian failure, hypothalamic dysfunction, pituitary dysfunction, and other endocrine abnormalities.

Female infertility may present as amenorrhea, the absence of menstrual periods that is usually precipitated by the failure of normal cycle estrogen production as evidenced by decreased estrogen levels. Causes of amenorrhea include pregnancy, uterine or endometrial abnormalities (congenital anomalies and intrauterine adhesions), ovarian failure (hypergonadotropic hypogonadism, hypogonadotropic hypogonadism, menopause, and hyperprolactinemia), chronic anovulatory syndromes, and hyperandrogenism (ovarian or adrenal). Other factors related to amenorrhea include excessive exercise, obesity, trauma, infection, tumors, stress, and hypo- and hyperthyroidism.

The clinical assessment of amenorrhea is dependent upon the woman’s age and her symptoms. In older women, the initial assessment involves menopause evaluation. In younger women of childbearing age, a serum human chorionic gonadotropin (hCG) test should be performed to rule out pregnancy as the cause of amenorrhea. Serum prolactin testing is performed to assess hyperprolactinemia as the cause of the amenorrhea. Additional assessment includes serum LH and FSH. Decreased levels of LH and FSH with decreased sex steroid levels are consistent with hypogonadotropic
hypogonadism; the defect is at the level of the pituitary or hypothalamus. When LH is abnormally elevated relative to FSH, polycystic ovary syndrome is the most likely cause of the amenorrhea. In women <30 years of age, a karyotype is done to rule out Turner’s syndrome. With evidence of androgen excess, such as hirsutism and/or virilization, serum total and free testosterone, dehydroepiandrosterone sulfate (DHEAS) and 17-hydroxyprogesterone are assayed. Additionally, thyroid stimulating hormone (TSH) and free thyroxine (FT_{4}) are measured if thyroid disease is the suspected causative agent in amenorrhea.

**Menopause**{1,2,4,12,13}: Menopause is the cessation of cyclic ovarian function that usually occurs in the fifth decade. The signs and symptoms of menopause result from the waning of ovarian follicular activity and thus decreased estradiol production. During the perimenopausal transition, menstrual cycles become irregular, usually shortened, and may be ovulatory or anovulatory. Follicle-stimulating hormone levels are increased, while LH is normal and estradiol and progesterone are decreased when compared to normal ovulatory cycles. With true menopause, both FSH and LH are greatly increased with a FSH:LH ratio >1. Follicle-stimulating hormone, usually >40 mIU/mL, is consistent with menopause related to primary ovarian failure. Estrogen levels are markedly reduced, and androgen levels are only slightly decreased.

**Hirsutism**{1,2,4,8,12,13}: Hyperandrogenemia in females causes hirsutism. Hirsutism is an abnormal androgen effect defined as excessive hair growth in females in androgen-responsive skin zones that are typically considered masculine in distribution. This excessive hair growth is caused by the metabolism of androgens locally at the hair follicle. The source of this often-familial hyperandrogenemia is the ovaries, adrenals, or peripheral tissues. The major causes of hirsutism include polycystic ovarian disease, Cushing’s syndrome, androgen-secreting tumors (adrenal or ovarian), the administration of anabolic steroids, increased peripheral sensitivity to androgens, increased peripheral production of androgens, and congenital adrenal hyperplasia (CAH—defective androgen synthesis, for instance 21-hydroxylase deficiency, 11-β-hydroxylase deficiency, 3-β-hydroxysteroid dehydrogenase deficiency).

Testing to elucidate the androgen source includes total and free testosterone, DHEAS, LH, FSH, SHBG, 17-hydroxyprogesterone, dihydrotestosterone, androstenediol, and androstenedione. Increased DHEAS levels, >7,200 ng/mL cut-off, point to the adrenals (>99% of adrenal origin) as the source of the excess androgens, while an increased level of testosterone, >200 ng/mL cut-off, implicates the ovaries (20% of ovarian origin). If virilization is also present, a tumor is suspected as the androgen source.

**Adrenogenital Syndromes**{7,9,12,13}: The adrenogenital disorders, inherited as autosomal recessive traits, cause either the absence or deficiency of 1 of the enzymes involved in the synthesis of the adrenal steroid hormones. Which enzyme is deficient determines which adrenal hormone class(es) is (are) diminished. In adrenogenital syndromes, cortisol production is decreased and ACTH is increased. Stimulation by adrenocorticotropic hormone (ACTH) causes congenital adrenal hyperplasia (CAH) and synthesis of hormones not requiring the deficient enzyme. The severity of the clinical presentation varies depending on the level of enzyme deficiency; complete absence of an enzyme is incompatible with life. Deficiencies of 21-hydroxylase and 11-β-hydroxylase cause increased androgen levels. Genetic females with a 21-hydroxylase deficiency have ambiguous genitalia resulting from the high circulating androgen concentrations. Those females with mild deficiency present with hirsutism after puberty. A 3-β-hydroxysteroid dehydrogenase deficiency causes decreased levels of estrogens and most androgens.

**Polycystic Ovarian Disease**{1,2,4,12,13}: Polycystic ovarian syndrome (Stein-Leventhal syndrome) is characterized by excessive androgen production by either the adrenals or the ovaries, which become enlarged and contain multiple cysts. Women with this disorder have menstrual irregularities, obesity, and hyperandrogenemia. Though the reproductive axis is intact, gonadotropin secretion by the pituitary is disturbed, resulting in a LH:FSH ratio >3.0.

**Infertility Clinical Presentations Affecting Both Sexes**

**Delayed Puberty**{4,13}: Delayed puberty is defined as failure to exhibit breast development by age 13 years in females or failure to have testicular enlargement by age 13.5 in males. Causes of delayed puberty are classified as systemic disorders, hypothalamic dysfunction, pituitary dysfunction, or gonadal dysfunction.

**Hyperprolactinemia**{1,2,8,11,13}: Increased levels of prolactin result in hypogonadism because of the hormone’s ability to inhibit pituitary LH and FSH release. As a result, estrogen levels are low in females and testosterone levels are decreased in males. The causes of increased serum prolactin can be classified as physiological (pregnancy; lactation; and acute release due to stress, hypoglycemia, orgasm, sleep, protein meals, nipple stimulation, anaerobic exercise, and seizures) or nonphysiological (pituitary tumor, hypothalamic disease, pituitary stalk compression, chronic renal failure, and certain medications).

A prolactinoma (pituitary adenoma) is a tumor that produces excessive amounts of prolactin and is the predominate cause (frequency 70% to 80%) of hyperprolactinemia. Females with a prolactinoma have amenorrhea, galactorrhea, and infertility, while adult males exhibit impotency, decreased libido, and infertility. Serum prolactin is used clinically to assess infertility and to diagnose and monitor prolactinomas. If the serum prolactin is >50 ng/mL, pituitary imaging techniques are performed. T4 shows serum prolactin levels at initial assessment and the probability that the initial result is diagnostic of a prolactinoma.
Other Pituitary Tumors\textsuperscript{5,8}: Besides the prolactinomas, pituitary tumors can also secrete bioactive intact gonadotropins or inactive free subunits -\(\beta_{FSH}\), \(\beta_{LH}\) or \(\alpha\).
Approximately 20\% to 25\% of pituitary adenomas are derived from gonadotropes. The persistently high levels of these inactive or active gonadotropins cause a clinical picture of hypogonadism.

Hyperthyroidism\textsuperscript{1-3,6,10}: Hyperthyroidism, or thyrotoxicosis, is defined as an increased concentration of the thyroid hormones. Symptoms of this disorder include nervousness, sweating, heat intolerance, moist and warm skin, tremor, angina, tachycardia, and menstrual irregularities.

Causes of hyperthyroidism include autoimmune disease (Graves’ disease) and toxic adenoma(s). Of these, Graves’ disease is the major cause of hyperthyroidism, constituting about 80\% of all hyperthyroid cases. The disease predominately occurs in females with an age of onset usually between 20 to 40 years.

Hypopituitarism\textsuperscript{2,3,6,10,13}: Diminished levels of the thyroid hormones characterize hypothyroidism. Symptoms of this disorder include lethargy, slow speech and thought, weakness, fatigue, dry and cold skin, cold intolerance, hypercholesterolemia, constipation, weight gain, hoarseness, bradycardia, and menstrual irregularities.

The incidence of hypothyroidism increases with age, with up to 5\% of the population older than 65 years having this condition, and occurs predominately in females. Causes of hypothyroidism are varied and include autoimmune disease, congenital disorders, treatment of Graves’ disease, and iodine deficiency, among others. The major cause of hypothyroidism is Hashimoto’s thyroiditis, an autoimmune disorder known also as chronic lymphocytic thyroiditis.

Primary hypothyroidism, in which the production of thyroid hormones is decreased and TSH production is increased, adversely affects menstruation because the increased levels of TSH, which has considerable alpha subunit homology to LH and FSH, negatively feed back to inhibit GnRH release.

Hypopituitarism\textsuperscript{1-8,12}: Hypopituitarism is initially recognized clinically as the failure of target organs to produce adequate levels of their hormones. Symptoms and signs include adrenal insufficiency, hypothyroidism, amenorrhea, impotency, hypoglycemia, and postpartum failure to lactate. Children with hypopituitarism often present with short stature and delayed puberty. Further investigation reveals inappropriately low levels of respective pituitary hormones, confirming the diagnosis. Deficiency of the pituitary hormones may be selective, partial, or total.

Causes of hypopituitarism include tumors (adenoma, craniopharyngioma, and meningioma), infiltration (hemochromatosis and sarcoidosis), infarction (post-partum pituitary infarction - Sheehan’s syndrome), inflammation (lymphocytic hypophysitis and infections), vascular (aneurysm, cavernous sinus thrombosis, and pituitary stalk compression), functional (psychosocial dwarfism, anorexia nervosa, severe malnutrition, and extreme exercise), and congenital.

Hyperandrogenism\textsuperscript{7,12,13}: The clinical presentation of hyperandrogenism is varied, ranging from ambiguous genitalia in the newborn female; premature puberty in the male child; gynecostasia, infertility and impotence in the adult male; hirsutism, oligo/amenorrhea, and acne in the adult female with mild hyperandrogenism; and altered body habitus, male-pattern balding, cliteromegaly, and deepened voice in the adult female with severe hyperandrogenism. The differential diagnosis of androgen excess may be difficult because of the myriad of causes of androgen excess. The battery of laboratory analytes for the differential diagnosis of hyperandrogenism include testosterone, free testosterone, SHBG, DHEAS, DHEA, androstenedione, androstenediol, dihydrotestosterone, and 3β-androstenediol glucuronide.

Testosterone is synthesized primarily by the testes in the male and the ovaries in the female. About a third of female testosterone is adrenal in origin. Testosterone is used to evaluate hirsutism and virilization in the females. The measurement of SHBG, the major serum testosterone carrier protein, may be important if a binding abnormality is suspected. Increased SHBG occurs with increased estrogen and hyperthyroidism, as well as with androgen deficiency; low SHBG is seen with decreased estrogens, androgen excess, hypothyroidism, acromegaly, obesity, malnutrition, and severe liver disease. Androstenedione, androstenediol, and DHEA are weak androgens synthesized equally by adrenals and ovaries. These androgens derive their androgenic effects through their subsequent conversion to testosterone. DHEAS, the sulfated conjugate of DHEA, is produced solely in the adrenals and thus serves as a marker of adrenal androgen synthesis; 99\% of DHEA circulates as DHEAS. The primary use of DHEAS is as a measure of adrenal androgen production. Dihydrotestosterone (DHT) is a metabolite of testosterone created by the action of 5α-reductase in peripheral tissues. 3β-androstenediol glucuronide is a metabolite of DHT and correlates well with 5α-reductase activity.

Cushing’s syndrome and disease\textsuperscript{9,13}: The clinical presentation of Cushing’s syndrome is caused by excessive amounts of circulating adrenal hormones, most notably, cortisol. Clinical features include truncal obesity, “moon face,” bruising, muscle weakness, poor wound healing, glucose intolerance, osteoporosis, acne, and hypertension. The effects of the
increased sex steroids from ACTH stimulation of the adrenals cause amenorrhea and hirsutism in females and impotence and gynecomastia in males. Causes of endogenous Cushing’s syndrome include an ACTH-producing pituitary adenoma, a glucocorticoid-producing adrenal neoplasm or an ectopic ACTH-producing neoplasm. Exogenous sources of glucocorticoids originate most commonly from chronic long-term glucocorticoid therapy.

**Addison’s disease**\(^6,13\): Primary adrenal insufficiency results from the destruction of entire adrenal cortex, either acute or chronic, thus causing deficiency of all adrenal steroids. Causes of acute adrenal destruction include adrenal hemorrhage, infection, uncontrolled anticoagulant therapy, and adrenalectomy. Chronic primary adrenal insufficiency, a rare disorder known as Addison’s disease, is most often caused by the autoimmune-mediated destruction of the adrenal cortex. Addison’s disease typically has a slow presentation of symptoms, the severity of which is inversely related to the amount of functioning gland remaining. Other causes of adrenal insufficiency include tuberculosis, metastatic carcinoma, and metabolic disorders such as amyloidosis. The progressive loss of the adrenocortical hormones, most notably cortisol, aldosterone, and the adrenal sex hormones, causes the clinical features of Addison’s disease, which include weakness, weight loss, dehydration, postural hypotension, and salt cravings. The reproductive manifestations, related to sex steroid loss, include infertility and the loss of body hair in adults and the delayed development of secondary sex characteristics in children.

**Testicular Feminization Syndrome**\(^2,3\): In testicular feminization syndrome, the phenotypic female has a 46 XY karyotype. The testes, which are present instead of ovaries, secrete testosterone. Unfortunately, the testes have end-organ resistance to testosterone and its metabolite, dihydrotestosterone. Because the testes also secrete excess estrogen and the gonadal response to this hormone is intact, female characteristics, such as female external genitalia and breast development, occur.

**Laboratory’s Role in Reproductive Endocrine Testing**

With health care cost containment, physicians must make diagnoses using a few select tests rather than the historical test panels. As a result, laboratory tests, the tools of diagnosis, must be more accurate and reliable than ever before. This is especially true for esoteric tests, such as the endocrine assays, which typically are more technically complex than routine analyses.

**Endocrine Immunoassays**\(^2,3\)

Typically, the reproductive hormones are present in the bloodstream at very low concentrations. Because of this, most endocrine analytes are determined using immunoassays with sensitive labels, either enzymatic, radioactive, fluorescent, or chemiluminescent. In immunoassays, antibodies are employed to interact with the hormone antigen. It is important for these antibodies to be specific by interacting only with the compound of interest and having minimal cross-reactivity with undesired compounds. In endocrinology, antibody specificity can be a daunting task because of structural similarities within hormone classes, especially the steroid, thyroid, and protein hormones. For instance, the sex steroid hormones, which are synthesized from a common pathway originating with cholesterol, are very similar in structure. Antibodies are needed that can distinguish between these sex steroids that can differ from one another by as little as the presence or absence of a single atom, a ring double bond, or a side chain group. The protein hormones, FSH, LH, hCG, and TSH, consist of 2 subunits—alpha and beta. The alpha subunits of these 4 proteins have considerable homology to each other. The beta subunits, on the other hand, are immunologically distinct and confer biological activity to the hormones. Often, an immunoassay for these quaternary protein hormones involves the use of a 2-site antibody sandwich, with 1 antibody directed towards 1 subunit and the other directed against the other subunit.

Factors that determine the efficacy of a specific immunoassay antibody to detect the analyte of interest include antibody variability, antibody avidity, and antibody epitope recognition. Antibodies used in immunoassays are classified as either monoclonal or polyclonal. Monoclonal antibodies have identical interactions, including recognition and binding strength, with a specific antigen because they are structurally the same. Because of their heterogeneous structural diversity, polyclonal antibodies may differ in their epitope recognition and binding avidity with the same hormone. Different antibodies directed against the same antigenic site can vary in the strength, or avidity, of their interactions with hormones. Likewise, different immunoassay antibodies can be directed against different antigenic sites on the same hormone, thus potentially producing different results, especially if the hormone itself is structurally diverse. In addition, cross-reactivity with unwanted molecules, such as hormone metabolites or endocrine therapeutics, also affects antibody specificity.

Other confounding issues include variability in the hormones themselves and their metabolites and assay standardization and configuration. Protein hormones undergo posttranslational modification, which creates a family of heterogeneous compounds with varying degrees of biological activity. This heterogeneity further increases the difficulty in achieving antibody specificity to the analyte of interest. Depending on the epitope to which the antibody is directed and the avidity with which the antibody binds to its epitope, different immunoassays can give different results with the same specimen. Likewise, different immunoassay standardizations can also produce discordant results. Different immunoassays calibrated against different standard preparations could give different results with the same
Immunoassay Performance

As laboratory professionals, we must fully understand all aspects of our diagnostic tools, especially assay strengths and weaknesses. Factors which assist in determining the clinical utility of an assay include assay accuracy, precision, reference interval, reportable range, analytical sensitivity, and analytical specificity. Information regarding these attributes is determined primarily through the method validation process. Additional information about assay performance can be obtained from the manufacturer’s product insert as well as from the published scientific literature.

The accuracy, or lack of bias, of an immunoassay is important for diagnostic purposes. Because of immunoassay issues, accuracy is often very difficult to ascertain. Inspection of proficiency survey results illustrates the tremendous variability that different immunoassays can produce for the same challenge. Oftentimes, a patient is serially monitored over time. For these patients, consistency between testing methodologies, which is related to assay accuracy, becomes vital. Bias between methods could lead to a diagnostic dilemma whereby the physician must determine whether the change in results is either analytical or biological.

It is important for any assay to yield reproducible results, and whether or not an assay can do that is determined by its precision. The precision of an endocrine assay is especially important near its analytical sensitivity, particularly if a medically diagnostic decision is made near the lower end of the reportable range. Since most immunoassays are designed to have optimal precision at the assay midpoint, the measurement extremes, especially the lower end, are left with poor reproducibility. Many manufacturers do not provide precision data near the assay’s analytical sensitivity limit because they know that assay performance is marginal there. To further complicate the issue, quality control material may not be readily available to monitor assay performance near the lower end of the reportable range.

The reference interval is the limits for laboratory results that define a patient population without disease. For hormones, the reporting of the appropriate reference interval is vital. Because many hormones have very complicated reference intervals, it is important to provide the physician with appropriate ranges so the correct diagnosis can be made. Many hormones, such as prolactin and cortisol, exhibit a diurnal variation. Several hormones, such as the reproductive hormones, have reference intervals that vary with sex, age, and menstrual status.

The assay reportable range is the set of results that can be reliably reported without dilution. Because dilutions introduce additional error, both analytical and mathematical, into any result, assays with expanded reportable ranges are often desired. In increasing the reportable range, the performance of the assay at its lower limit usually suffers.

Understanding an assay’s analytical sensitivity is important for those endocrine assays that have a diagnostic decision point near the assay’s lower limit of its reportable range. The assay’s analytical sensitivity, or its ability to detect small quantities of the measured analyte, is determined as either the limit of detection or the limit of quantification. The limit of detection, which is the smallest analyte concentration that can be distinguished from zero, typically yields lower values than those derived from the limit of quantification, which is the minimum concentration whose imprecision is known within some required limit of error. For those assays, such as high sensitivity TSH, where low results are diagnostically significant, the limit of quantification is the preferred method for determining analytical sensitivity.

Analytical specificity, the ability of a method to determine only the analyte it is supposed to measure, is assessed through interference studies, particularly with lipemia, icterus, and hemolysis, the major interferences found in serum and plasma biological specimens. Cross-reactivity experiments are important when compounds are present that could interact with antibodies because of structural similarity to the analyte of interest.

Endocrine Preanalytical Issues

Specimen collection is a factor that must be considered to achieve appropriate test results. Many hormones are released in a pulsatile fashion. In some cases, these spikes can be exaggerated by stress. Depending on the timing of collection and the occurrence of the pulsatile spikes, an abnormally increased result could be obtained.

For hormones with diurnal variations, specimens must be collected at times for which a reference interval exists. Note that these reference intervals assume that the patient has a normal sleep-wake cycle.

Test Interpretation

Often, endocrine results are interpreted in relationship to one another and the patient’s physiological state. For instance, in a woman suspected of being postmenopausal, LH and FSH are compared to her estradiol levels and her menstrual history.

Discordant Results

No matter how carefully we select our endocrine immunoassays, we still may be faced with discordant results, that is, results that do not match the patient’s clinical picture. Detection of discordant results can be accomplished by analyzing the specimen by another method, analyzing the analyte in a different biological fluid if appropriate, performing dilutional studies, or by completing serial measurements.

Conclusion

Infertility is a common disorder today. Identification of the complex causes of infertility is important for appropriate treatment. The physician relies on the patient’s physical presentation as well as the laboratory results to make the correct diagnosis and to design the appropriate intervention.

2. Cook JD, Noel S. Special topics in endocrinology: Thyroid, infertility and malignancies, ASCP, Chicago, IL, April 2002.


