



# A Pragmatic Approach to Hormonal Testing in the Assessment of Disorders of Female Reproduction

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### Abstract

**Objective:** To explore the value and limitations of various hormonal assays used to investigate disorders of female reproduction.

**Sources of information:** We performed a literature search using keywords on OvidSP Medline and reviewed the most recent clinical practice guidelines and position statements published by relevant societies including the American Society of Reproductive Medicine, the Endocrine Society, the Society of Obstetricians and Gynecologists of Canada and the American Congress of Obstetricians and Gynecologists

**Main message:** When approaching a female patient with concerns about her reproductive health, it is important to first consider how a given hormonal test will guide diagnosis, impact treatment or define prognosis. Sometimes, the interpretation of hormone levels can cloud the clinical diagnosis. Careful consideration of the value and limitations of each test helps to streamline the path to diagnosis and the cost to the healthcare system. We review the utility of various hormone tests in the context of two cases of primary infertility: the ovulatory patient and the anovulatory patient.

**Conclusion:** Understanding the physiology of each reproductive hormone, their interactions and their impact on the hypothalamic-pituitary-ovarian axis, as well as the limitations of the currently available hormonal assays can help clinicians choose wisely when investigating women with reproductive dysfunction.

### Introduction

The hormonal environment responsible for female reproduction is in constant flux within each menstrual cycle and over the course of a woman's reproductive lifetime. It is this cyclicity that makes defining and interpreting clinically relevant serum hormone parameters a challenging task. When approaching a patient with concerns about her reproductive health, it is important to first consider how a given hormonal test will guide diagnosis, impact treatment or define prognosis. Sometimes, the interpretation of hormone levels can cloud the diagnosis because of innate limitations with the hormonal assay, scheduling difficulties with the test (phase of menstrual cycle) or a lack of an appropriate normal range for the patient population.

In this article, two cases of primary infertility, in the ovulatory and anovulatory patient, will demonstrate a pragmatic approach to hormonal testing in women attempting to conceive. Reporting reference ranges has been intentionally avoided whenever possible

because laboratory assays and units of measurement vary among centers. It is important to refer to one's own laboratory reference ranges when interpreting results.

### Case 1: Primary Infertility in the Ovulatory Patient

A 32 y/o G0P0 presents with primary infertility, clinically defined by the World Health Organization as 1 year of regular unprotected intercourse [1]. A thorough history reveals regular menstrual cycles and frequent intercourse without the use of lubricant. There is no history of pelvic inflammatory disease, pelvic surgery and no symptoms of endometriosis. On physical examination, the patient is of normal body habitus without signs of hyperandrogenism, thyroid dysfunction or hyperprolactinemia. Both abdominal and pelvic examinations are normal. Her partner is healthy and semen analysis is normal. In this case, assuming that there are no female structural abnormalities or male factor contributing to their infertility, the two goals of hormonal testing are to confirm ovulation and assess ovarian reserve.

### Confirmation of ovulatory function

Ovulatory disorders occur in approximately 21% of infertile couples, so it is important to ensure ovulation in any woman presenting with concerns about fertility [2]. A history of regular menses every 24-35 days, with predictable flow and pre-menstrual molimina predicts ovulation in 97% of cases [3]. A mid-luteal serum progesterone retrospectively confirms ovulation by testing for the presence of progesterone produced by a corpus luteum. Progesterone is produced in the corpus luteum after ovulation and levels peak 7 days later, so the best day to test can be calculated by subtracting 7 from the predicted cycle length, assuming a relatively constant 14 day luteal phase. The absolute value of progesterone does not predict the "quality" of the luteal phase, because progesterone is dependent on LH pulsatility and concentrations can vary up to 8-fold in a 90 minute period [4,5]. Another indirect measure of ovulation is through urinary LH detection, and kits can be purchased for home use [6]. Urine LH is not as reliable as serum progesterone for confirming ovulation because of false positive and negative or uninterpretable results [7]. However, these kits are useful for timing intercourse during the optimal "window" just before ovulation [5]. A Cochrane review was published in March 2015, which found that although there is "insufficient evidence to draw definite conclusions,...the findings suggest that timed intercourse [with urinary LH kits] is

associated with higher pregnancy rates...but the benefit is small” (14-23% vs 13% reported pregnancy rates) [8].

Of note, basal body temperature charting has been shown not to be a reliable measure for retrospectively detecting ovulation and therefore is not recommended [5,9,10]. Furthermore, body temperature rises as a result of increasing progesterone, which occurs *after* ovulation. Progesterone also makes the cervical mucus impenetrable to sperm, so any shift in temperature is too late to inform decisions about timing of intercourse in the current cycle.

It is well established that an elevated prolactin (PRL) or thyroid dysfunction can disrupt ovulation. However, in otherwise asymptomatic ovulatory women presenting with unexplained infertility, screening for pituitary hormone level abnormalities is controversial [11,12].

There is good evidence that the incidence of hyperprolactinemia in infertile but ovulatory patients does not appear to be higher than the baseline population risk [13,14]. Furthermore, among normally menstruating women with elevated prolactin levels, no differences have been found in mid-luteal progesterone levels or conception rates. Therefore, screening for hyperprolactinemia in regularly menstruating women without galactorrhea is not recommended [12,15].

Testing and treating thyroid dysfunction among regularly menstruating women is heavily debated. The generalizability of the literature to this unique population is limited by varied TSH cutoff values and a lack of control group in retrospective study designs [16,17]. Furthermore, results of studies that assess thyroid function in early pregnancy should not be extrapolated to the infertile population. hCG weakly stimulates the thyroid because of the shared alpha subunit with TSH [18]. Thus, TSH levels transiently decrease in the first trimester of pregnancy. Therefore, the TSH cutoff in early pregnancy cannot be used as a reference for TSH levels in non-pregnant women, when hCG is not yet present.

A recent guideline published by the American Society of Reproductive Medicine highlights the difficulties in formulating recommendations based on current data, but concludes that women with infertility may be considered at increased risk of subtle thyroid dysfunction and there is fair evidence to recommend screening with TSH [17]. If the TSH level is found to be > 2.5mIU/L but < 4 mIU/L and it is unclear whether to treat or not, there is now fair evidence to consider screening for thyroid antibodies. If positive, levothyroxine treatment in this subgroup of patients may improve pregnancy outcomes [17]. In other words, at TSH levels between 2.5-4 mIU/L clinicians may choose to initiate low dose levo-thyroxine supplementation, or choose only to treat if the patient is also found to have thyroid antibodies.

In summary, a patient history that endorses a menstrual pattern that is predictable in interval, duration and flow and associated with symptoms of premenstrual moodiness or dysmenorrhea is highly predictive of ovulation. In this case, no further testing is necessary to confirm ovulation. However, if the history is less clear, a mid-luteal progesterone to confirm ovulation is more reliable than urine LH detection or basal body temperature charting.

If the patient is ovulatory and otherwise asymptomatic, prolactin testing is not necessary. Thyroid testing in ovulatory but infertile patients is reasonable because correction of subclinical hypothyroidism may improve pregnancy outcomes.

### Assessment of ovarian reserve

An overall trend towards delayed childbearing among women increases the risk of infertility due to a reduction in both oocyte quantity and quality often referred to as decreased ovarian reserve (DOR) [19,20]. As the field of assisted reproductive technologies (ART) grows, so does the desire to find a test of ovarian reserve that helps predict how a woman will respond to treatment and achieve a live birth [21]. Age is the most accessible surrogate for decreased

ovarian reserve and remains the most important factor in predicting successful pregnancy in regularly cycling women [22,23]. However, a number of hormonal tests exist for assessment of oocyte quantity which, when coupled with age, attempt to predict reproductive potential.

Follicle stimulating hormone (FSH) is the oldest hormonal test of oocyte quantity. FSH is stimulated by pulsatile GnRH and inhibited through negative feedback by inhibin B and estradiol [24]. As the follicular pool decreases with advanced age, the concentration of inhibin B decreases, resulting in loss of negative feedback and rise in FSH [25]. FSH is most appropriately measured in the early follicular phase (day 3-5), when levels are generally highest before suppression by rising estradiol levels [24]. Therefore, estradiol should be ordered with FSH to ensure that a premature elevation in estradiol has not masked what would be an otherwise elevated FSH.

The main disadvantages of FSH as a test of oocyte quantity are:

1. FSH is secreted in a pulsatile fashion (every 3 hrs), so there can be great intra- and intermenstrual variability in results [26].
2. Among regularly cycling women, FSH is only accurate in predicting poor response to assisted reproductive technologies at very high threshold levels. At such high levels, the sensitivity of the test is poor, and therefore only identifies a small proportion of women who may in fact have diminished ovarian reserve [27]. In other words, high FSH levels tell us ovarian reserve is poor, but normal levels are not informative. This limitation greatly diminishes the clinical usefulness of FSH testing.

Anti-mullerian Hormone (AMH) has now emerged as the most useful test of oocyte quantity. AMH is produced by granulosa cells surrounding preantral and early antral follicles [25] and thus, serum levels correlate with the pool of developing follicles within the ovaries [28,29]. Some advantages of AMH as a test of ovarian reserve are:

1. AMH is expressed independently of GnRH (not pulsatile) and can be measured at any point in the menstrual cycle without significant variability [30-33].
2. AMH is largely unaffected by hypothalamic or pituitary disorders such as hypothalamic amenorrhea or hyperprolactinemia [29].
3. AMH is more sensitive than FSH at identifying a loss in ovarian reserve after gonadotoxic injury, such as chemotherapy or radiation [28,34].

However, the limitations of AMH that cannot be overlooked include:

1. A lack of international standard interpretation for AMH levels. Up until recently, two different commercial laboratory enzyme-linked immunosorbent assays (ELISA) existed [35]. There has now been consolidation into a single assay called the Beckman assay, which will help standardize research [29,36].
2. There are limited data on the use of AMH to predict natural fertility. AMH clearly predicts ovarian response to controlled ovarian hyperstimulation and is useful in the setting of ART, but [28] there is no consensus on the AMH value below which fertility potential is definitely reduced [37,38].

Other hormonal tests of ovarian reserve, such as inhibin B and estradiol alone have proven to be inferior to FSH and AMH and therefore, should not be used to test ovarian reserve [26,27].

It is worth mentioning that a sonographic measure of ovarian reserve, known as the Antral Follicle Count (AFC), is frequently used in combination with serum FSH and/or AMH values to predict ovarian response to gonadotropin stimulation for the purposes of in vitro fertilization. The AFC is the total number of visible follicles seen in both ovaries on transvaginal ultrasound in the early follicular phase. However, just as is the case with hormonal parameters, a low AFC does not “reliably predict failure to conceive” [39,29].

**Table 1:** Utility of various hormonal tests for primary infertility in the ovulatory patient.

UTILITY	TEST	INDICATION
HIGH Utility	Patient age	Remains the best predictor of success of pregnancy through natural conception or Assisted Reproductive Technologies
	Anti-Mullerian Hormone	The most reliable screening test for poor ovarian response to ovarian stimulation
LOW Utility	Early follicular FSH with estradiol	A specific but not sensitive test of decreased ovarian reserve
	Urinary LH	Useful if couple has difficulty with timing or frequency of intercourse
	TSH	To rule out subclinical hypothyroidism
	Thyroid antibody testing: If TSH mildly elevated	To help determine whether or not to initiate treatment in patients with subclinical hypothyroidism
	Mid-luteal progesterone	Not necessary to confirm ovulation if the patient reports a history of regular and predictable menses
NO Utility	Prolactin	
	Inhibin B	
	Estradiol alone	

In summary, AMH is the single best test for assessment of ovarian reserve, but interpretation is still limited by a lack of international standard and inter-assay variability. Cycle day 3 FSH with estradiol is useful if elevated in predicting poor ovarian reserve, but not helpful when the level is within normal range. Inhibin B and estradiol alone are not helpful for assessing ovarian reserve.

Table 1 outlines the hormone tests that may be considered in this first case of a regularly menstruating woman presenting with primary infertility.

### Case 2: Primary Infertility in the Anovulatory Patient

A 28 y/o G0P0 presents with unpredictable and irregular menses that are often prolonged and heavy but painless. On history, she has a tendency to gain weight easily and endorses unwanted hair growth over her face and abdomen and persistent acne on her chest and back.

Distinct from the first case, here we are presented with a patient with clinical features of hypothalamic-pituitary-ovarian axis dysfunction, resulting in an- or oligo-ovulation and androgen excess.

Polycystic Ovarian Syndrome (PCOS) is the most common female endocrine abnormality and is “a collection of signs and features, where no single test is diagnostic. In essence, the whole (or global assessment) is greater than the sum of the individual parts (or features)” [40]. As such, the patient should be viewed as her own “bioassay” to help make the diagnosis, rather than relying on hormonal assays alone [41]. The 2003 Rotterdam criteria for the diagnosis of PCOS validates this approach, requiring two out of the following three diagnostic criteria to make the diagnosis of PCOS [42]:

- 1) Clinical and/or biochemical signs of hyperandrogenism
- 2) Oligo or anovulation
- 3) Polycystic ovaries (by ultrasound)

The diagnosis of PCOS requires the exclusion of other disorders of androgen excess in women, including congenital adrenal hyperplasias (CAHs) and androgen-secreting neoplasms (ASNs). There are also a number of disorders that may result in ovulatory dysfunction, such as premature ovarian failure, hyperprolactinemia and thyroid dysfunction that also need to be ruled out before making the diagnosis of PCOS [40,43,44].

Thus, when selected appropriately, hormonal assays can help to both support the diagnosis of PCOS and rule out other endocrine disorders that can present with similar features of either clinical hyperandrogenism or ovulatory dysfunction.

### Hyperandrogenism

Clinical hyperandrogenism can present as hirsutism, acne and androgenic alopecia. Of all these features, hirsutism or unwanted hair growth is most strongly associated with PCOS (70-80%), followed by persistent acne (20-40%) and alopecia (10%) [40].

Biochemical hyperandrogenemia can be more difficult to define than clinical hyperandrogenism because of innate limitations in androgen testing in women.

Androgens arise from 3 sources in the female: the ovaries, the adrenals and the peripheral compartment (adipose tissue). Testosterone, the most potent androgen is mainly produced by the ovary and through peripheral conversion from androstenedione. Conversely, 95% of dehydroepiandrosterone sulfate (DHEA) is produced by the adrenal glands. Therefore, some clinicians consider testing Testosterone and DHEA levels as a way of differentiating between PCOS and adrenal causes of hyperandrogenism. However, the sex steroid hormone production pathway is complex and multi-directional, so an over-reliance on hormone testing can in fact cloud what would otherwise be a straightforward clinical diagnosis.

### Testosterone

Sixty-six percent of testosterone is bound to sex hormone binding globulin (SHBG) and 33% is bound to albumin, leaving only 1-2% of testosterone unbound. It is this unbound, or bioavailable testosterone (bioavailable T) that enters target tissues [45]. However, measurement of bioavailable T has significant limitations. The gold standard for testing is through equilibrium dialysis, where the unbound testosterone travels through a membrane with microperforations only large enough for the small, unbound molecules [46]. Although accurate, equilibrium dialysis is technically complex and extremely expensive. Instead, analogue-based radioimmunoassays, which use iodine-labeled analogue tracers to compete with testosterone, are commercially available and widely used. The results however, are highly inaccurate because the tracer can bind to other proteins as well [47]. Lastly, bioavailable testosterone can be indirectly calculated by concomitantly measuring total testosterone and SHBG. Although SHBG testing is simple and reliable, total T measurements are still variable among laboratory assays, especially at the lower ranges found in most women. Finally, a normal reference range for women is not well-defined in the PCOS population, as there lacks a large normative database of androgen levels for women [48].

In addition to all of these limitations of testosterone assays, interpreting serum T levels is further complicated by the intracrine physiological action of testosterone at the cellular level. Testosterone can be converted to either dihydrotestosterone or estradiol. Tissue sensitivity to androgens will vary according to the amount and activity of enzymes such as 5alpha-reductase and aromatase. There is considerable variability between individuals in enzymatic activity, which can not be correlated with serum T levels [48]. In summary, testing total testosterone levels in females is unreliable and not recommended. Direct or indirect measurement of free/bioavailable testosterone can be performed, but is less valuable than the careful clinical assessment of hyperandrogenism [49]. The limitations of direct testosterone immunoassays for clinical use, particularly for low concentrations found in women and children, have been recognized for some time. This problem prompted a The Endocrine Society position statement recommending either liquid chromatography/tandem mass spectrometry (LC-MS/MS) or immunoassay after extraction and chromatography for measuring testosterone in women and children [3]. Subsequently, our sister journal, The Journal of Clinical Endocrinology and Metabolism announced that new submissions starting 2015 must employ LC-MS/MS assays for sex steroids [4].

## DHEA/DHEA-S

DHEA is the primary androgen produced by the adrenals. Although PCOS is considered a condition of excess *ovarian* androgen production, elevated DHEA levels often correspond with increased circulating testosterone [40]. DHEA is a difficult hormone to measure because of diurnal variation, a wide normal range and spurious elevations from any sources of stress including phlebotomy itself. Instead, Dehydroepiandrosterone sulfate (DHEA-S), the longer acting metabolite of DHEA, is often measured instead. Although less variable, DHEA-S levels reflect not only adrenal production of DHEA levels but also the enzymatic activity of DHEA sulfotransferase [40]. Therefore, DHEA is neither a sensitive nor specific test for PCOS and should not be relied on as a criterion for diagnosis.

## LH/FSH ratio

Lutenizing Hormone (LH) directly stimulates ovarian theca cells to secrete androstenedione, the immediate precursor of testosterone, leading to hyperandrogenism [42]. In lean patients with PCOS, circulating LH levels can be elevated due to increased pulse frequency of GnRH and LH, while FSH levels are normal or even decreased. This results in a higher LH/FSH ratio in the follicular phase of the cycle. However, this elevation does not hold true in the obese PCOS population [40]. Furthermore, the cutoff ratios are dependent on the type of assay and units of measurement used [50].

In summary, basal gonadotropin (LH and FSH) levels are not useful in the diagnosis of PCOS, except to rule out Primary Ovarian Insufficiency [40].

## Oligo - or anovulation

Oligo or anovulation among women with PCOS is common and one of the three diagnostic features of the syndrome in all definitions of the syndrome that have been proposed by Rotterdam, National Institutes of Health, and the Androgen Excess PCOS Society. In a prospective study of unselected women, 60% of patients with PCOS were found to have ovulatory dysfunction, which usually presents as oligomenorrhea [40]. However, 15-40% of women with PCOS report normal cycle length (27-34 days), but are found to have ovulatory dysfunction based on a negative, appropriately timed progesterone test [40]. This emphasizes the importance of asking patients about premenstrual molimina and dysmenorrhea, which may be absent in this population.

## Polycystic ovaries

The presence of 12 or more antral follicles per ovary and/or increased ovarian volume of > 10cc meet the definition of polycystic ovaries [42]. However, these ultrasound findings are non-specific. In fact, as ultrasound technology improves resolution, polycystic appearing ovaries are commonly seen in normally ovulating women, so as an isolated finding, does not warrant further investigation [51]. More recently, studies are focusing on AMH as a test to replace the morphologic description of polycystic ovaries as part of the diagnostic criteria of PCOS [52]. There is good biologic plausibility that an elevated serum AMH reflects an increased antral follicle number, but more studies are needed before incorporating AMH into the diagnosis of PCOS [29].

## Rule Out Other Disorders of Oligo-Ovulation or Hyperandrogenism

### Pregnancy

Pregnancy should always be excluded in a reproductive aged woman presenting with new onset menstrual disturbance through urinary or serum beta-subunit hCG testing, regardless of the patient's perception of the likelihood of a possible pregnancy.

### Non-classic congenital adrenal hyperplasia

Non-classic congenital adrenal hyperplasia (NCAH) is an autosomal recessive disorder, almost always due to 21-hydroxylase

deficiency, which leads to defective cortisol production. In response, there is an increase in Adrenocorticotropic Hormone (ACTH), which leads to excess synthesis of precursor steroids (17-OH progesterone) and thus, excess adrenal androgen production (DHEA). Congenital adrenal hyperplasia affects 0.6-8.4% of women with hyperandrogenism and this prevalence varies significantly with ethnicity and is found to be more common among Ashkenazi Jewish, Mediterranean, Middle eastern and Indian populations [53,54]. When the enzymatic mutation is less severe, clinical symptoms of NCAH appear later in life and can be indistinguishable from PCOS. A large prospective study by Escobar-Moreale in 2008 showed that when a follicular phase 17-OH progesterone level is drawn in the morning, it has a 97% chance of correctly identifying women with NCAH among women who present with hyperandrogenism [53]. To confirm the diagnosis, only those patients with a positive screen should go on to have an ACTH stimulation test or genetic testing for gene mutations. DHEA and DHEA-S are downstream metabolites of 17-OH progesterone and in theory, levels should be elevated among patients with NCAH. However, studies have shown that there is significant overlap between levels among patients with and without NCAH [53,55]. Therefore, an elevation in DHEA-S is neither sensitive nor specific for Late Onset CAH.

In summary, 17-OH progesterone drawn in the morning during the follicular phase is a useful screening test to rule out NCAH, especially in at-risk ethnicities where the prevalence of disease is more common [56].

With a positive screen confirmatory testing should be ordered. DHEA-S testing is not as useful and should not be considered as part of the diagnosis of NCAH.

### Primary ovarian insufficiency (POI)

As discussed in the first case, markedly elevated FSH is a specific but not sensitive test. When the clinical suspicion of POI is high, including women with oligo- or amenorrhea, an FSH with estradiol is a very useful test [57,58]. POI is defined as "the triad of amenorrhea for at least 4 months, sex steroid deficiency [low estradiol] and two recordings of [elevated] serum concentrations of FSH" [57].

### Prolactin and thyroid dysfunction

Along with gonadotropins, dysregulation of prolactin (PRL) and altered levels of thyroid stimulating hormone (TSH) released from the anterior pituitary can lead to ovulatory dysfunction. High levels of PRL interfere with GnRH pulsatility and can impair normal ovulation. PRL assays are highly sensitive, specific and internationally standardized, but prolactin is released episodically, so levels vary through the day and can be spuriously elevated by triggers, including ingestion of certain foods, phlebotomy stress, sleep, and intercourse [59]. Therefore, one elevated prolactin level should be repeated fasting before making the diagnosis of hyperprolactinemia.

Ovulatory dysfunction is also present in an estimated 20% of women with thyroid disturbances (both hyper- and hypothyroidism) [60]. Similar to PRL, TSH hormonal assays have become highly sensitive and specific, with little cross reactivity with the other glycoprotein hormones that share alpha subunits (LH, FSH and hCG) [59]. Although TSH is also released episodically, the pulse amplitude is lower and the half-life is longer than PRL, resulting in less overall variability between measurements.

In summary, both TSH and PRL testing are recommended in patients presenting with ovulatory dysfunction.

### Other rare disorders

Fortunately, other rare endocrine disorders resulting in ovulatory dysfunction and hyperandrogenism can often be excluded by thorough history and physical examination [42,44]. Ovarian or adrenal Androgen Secreting Neoplasm (ASN) often present with rapid onset and progressive hyperandrogenism or overt virilization. Cushing's syndrome (ACTH-secreting tumors) may present with ovulatory dysfunction and hyperandrogenism but is also associated

**Table 2:** Utility of various hormonal tests for primary infertility in the patient with ovulatory dysfunction.

UTILITY	TEST	INDICATION
<b>HIGH Utility</b>	b-hCG	To rule out pregnancy
	Early follicular FSH and Estradiol	To rule out primary ovarian insufficiency
	PRL and TSH	To rule out hyperprolactinemia and thyroid dysfunction
	17-OH Progesterone drawn in the morning during the follicular phase	To rule out non-classic congenital adrenal hyperplasia in high risk populations (Ashkenazi Jewish, Mediterranean, Middle eastern and Indian)
	Mid-luteal Progesterone	To confirm ovulation if the menstrual history is unclear. Can be deferred if the patient has grossly abnormal cycle lengths
<b>LOW Utility</b>	Free/Bioavailable Testosterone, DHEA-S	To identify biochemical hyperandrogenism. However, androgen testing is neither sensitive nor specific for the diagnosis of disorders of androgen excess such as PCOS and NCAH
	Early follicular serum LH with FSH	To calculate the LH/FSH ratio as a marker of PCOS. This test is especially unreliable in obese patients with suspected PCOS.
	Growth Hormone, 24 hour urinary or serum Cortisol	Only recommended when other clinical features are suggestive of pathology
	Anti-mullerian Hormone	Not yet a validated test for the diagnosis of PCOS

with hypertension, proximal myopathy or other physical findings such as moon facies, rapid weight gain, bruising or thinning of skin. Acromegaly, an extremely rare condition that results from excess growth hormone (GH) often presents with coarsening of facial structures and enlargement of hand and foot size [61,62]. Guidelines agree that given the rarity of these conditions and the unique features that may differentiate them from PCOS, screening by history and physical examination may be adequate and hormone testing is only required in cases of high suspicion [40,44]. In summary, hormone testing for rare disorders is not required in the absence of suspicious clinical features.

Table 2 outlines the hormone tests that may be considered in this second case of a woman presenting with clinical hyperandrogenism, ovulatory dysfunction and primary infertility.

## Conclusions

Understanding the physiology of each reproductive hormone, their interactions and their impact on the hypothalamic-pituitary-ovarian axis, as well as the limitations of the currently available hormonal assays can help clinicians choose wisely when investigating women with reproductive dysfunction.

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