LABORATORY TESTS IN DIAGNOSIS OF FEMALE INFERTILITY

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• Couples wish to know why they have not been able to conceive and, depending on the etiology, to be provided with the most appropriate options available to them for treatment.

• For diagnosing and treatment we consider:

  1. Usefulness of any diagnostic test include sensitivity (to minimize false negative) specificity (to minimize false positive)
  2. Safety
  3. Cost
INDICATIONS AND TIMING OF EVALUATION

1. Initiate evaluation after 12 months of unprotected and frequent intercourse:
   - Women under age 35 years without risk factors for infertility.

2. Initiate evaluation after six months of unprotected and frequent intercourse:
   - Women age 35 to 40 years.
3. Initiate evaluation **less than six** months of unprotected and frequent intercourse:

- Women **over 40 years of age**.
- Women with oligomenorrhea/amenorrhea.
- Women with a history of chemotherapy, radiation therapy, or advanced stage endometriosis.
- Women with known or suspected uterine/tubal disease.
- Male partner has a history of testicular surgery, adult mumps, impotence or other sexual dysfunction, chemotherapy and/or radiation.
FEMALE INFERTILITY CAUSES

- Tubal and pelvic pathology: 40%
- Ovulatory dysfunction: 40%
- Unexplained infertility: 10%
- Unusual problems: 10%
WHO CLASSIFICATION OF ANOVULATION

WHO class 1: Hypogonadotropic hypogonadal anovulation (hypothalamic amenorrhea)

Low or low-normal serum FSH and low serum estradiol concentrations due to decreased hypothalamic secretion of GnRH or pituitary unresponsiveness to GnRH.

WHO class 2: Normogonadotropic normoestrogenic anovulation

FSH secretion during the follicular phase of the cycle is subnormal. This group includes women with PCOS. Some ovulate occasionally, especially those with oligomenorrhea.
WHO CLASSIFICATION OF ANOVULATION

WHO class 3: Hypergonadotrophic hypoestrogenic anovulation

The primary causes are POF (absence of ovarian follicles due to early menopause) and ovarian resistance (follicular form).

Hyperprolactinemic anovulation

hyperprolactinemia inhibits gonadotropin and therefore estrogen secretion; they may have regular anovulatory cycles, but most have oligomenorrhea or amenorrhea. Their serum gonadotropin concentrations are usually normal or decreased.
SCREENING TESTS

- Pap smear screening is recommended for all sexually-active women of reproductive age who have a cervix.
- A blood type, Rh factor, and antibody screening (in Rh-negative women) also are recommended, if not already known.
- Screening for cystic fibrosis (CF) be offered to individuals with a family history of CF, partners with CF, one or both partners are Caucasian or of Ashkenazi Jewish descent.
SCREENING TESTS

• All women attempting pregnancy with undocumented previous rubella infection or vaccination should be tested for immunity, and vaccinated if seronegative.

• Centers for Disease Control and Prevention (CDC) has determined that women need not avoid pregnancy for more than 1 month after vaccination.

• The CDC also recommends that all women without history of previous infection or evidence of immunity or vaccination against varicella (chicken pox) receive two doses of vaccine and avoid pregnancy for 1 month after each dose.
SCREENING TESTS

• Screening for sexually-transmitted infections (STI) is recommended for all women at moderate to high risk for infection.

• Screening for all pregnant women for chlamydia and gonorrhea (nucleic acid-based tests), syphilis (rapid plasma reagin; RPR), hepatitis B (hepatitis B surface antigen; HBSAg), and voluntary screening for human immunodeficiency virus type I (HIV-I) at the first prenatal visit.
SCREENING TESTS

• For women receiving inseminations of donor sperm, (ASRM) considers HIV-I screening mandatory, recommends screening for syphilis, hepatitis B and C, cytomegalovirus (CMV), HIV-2, and human T-cell lymphocyte virus (HTLV) types I and II, and suggests screening for chlamydia and gonorrhea.

• For male partners of women receiving inseminations of donor sperm, the ASRM recommends HIV-I and other STI screening.

• For recipients of donor oocytes or embryos and their male partners, the ASRM recommends screening for syphilis, hepatitis B and C, CMV, and HIV-I.
Prophylactic antibiotics should be considered before uterine instrumentation if screening has not been carried out. (D)-NICE

Before undergoing uterine instrumentation women should be offered screening for Chlamydia trachomatis (B)-NICE
OVULATORY FUNCTION

• Women who report monthly menses and molimina (breast tenderness, dysmenorrhea, bloating) are typically ovulatory.

• A woman with regular menstrual cycles every 21-35 days is most likely to be ovulating. But in a small percentage of cases (<10%) they may still be anovulating.
Assessment of ovulatory function
PROGESTERONE

• A progesterone level (mid-luteal) >3 ng/mL is evidence of ovulation. Serum progesterone measurement should be taken one week before the onset of expected menses (for example, day 21 if the woman has a 28-day cycle).

• If a woman has long and unpredictable cycles, the sample may need to be repeated weekly until the next cycle starts.
LH SURGE

- The LH surge appears in the urine within 12 hours after it appears in the serum, The rise in serum LH typically occurs approximately 36 hours before the oocyte is released from the follicle.

- Home kits have a 5 to 10 percent false positive and false negative rate.

- According to the WHO, regular use of urinary LH kits should be discouraged because of the psychological pressure of timing intercourse and the expense of the kits.
**ENDOMETRIAL BIOPSY**

- **Endometrial Biopsy** is preferably done between days 21 and 24 of the luteal phase of the cycle. For two reasons: (1) to document a secretory endometrium, which is indirect evidence that ovulation has occurred, and (2) to evaluate whether the maturity of the secretory endometrium is in phase (consistent with menstrual cycle date) or out of phase (luteal phase defect).

- A discrepancy of two or more days behind the menstrual dating defines luteal-phase deficiency (LPD).
ENDOMETRIAL BIOPSY

- In normal fertile women, half will have a single out-of-phase biopsy (using two-day or greater lag criteria). As the treatment of luteal phase defect does not improve pregnancy outcome in infertile women, luteal phase evaluation by histological dating of the endometrium is not worthwhile. (B)

- It is invasive, expensive, uncomfortable, unnecessary for evaluation of ovulation, and ineffective for assessment of endometrial receptivity for implantation.
• (BBT) chart has largely been abandoned because it is cumbersome and is not reliable as a predictor of the time of ovulation. (B)

• disappearance of the preovulatory follicle/follicles together with the appearance of free fluid in the pouch of Douglas confirms that ovulation has occurred.
Assessment of ovarian reserve
DAY 3 FSH AND CCCT

- Both the day 3 FSH level and the CCCT, which is a provocative test for measurement of FSH, are widely used for screening ovarian reserve.

- Women with a reduced pool of follicles and oocytes have insufficient production of ovarian hormones to provide normal inhibition of pituitary secretion of FSH, so FSH rises early in the cycle.
DAY 3 FSH AND CCCT

- The CCCT involves oral administration of 100 mg clomiphene citrate on cycle days 5 through 9 with measurement of day 3 and day 10 FSH levels and day 3 estradiol level.

- With either test, a normal result is not useful in predicting fertility, but a highly abnormal result (we use FSH >20 mIU/mL) suggests that pregnancy will not occur with treatment involving the woman's own oocytes.

- If CCCT is performed, we consider FSH less than 15 mIU/mL on both day 3 and day 10 suggestive of adequate ovarian reserve; an elevated FSH level on either day 3 or day 10 suggests decreased ovarian reserve.
Based on these findings and the cost advantage and simplicity of the day 3 FSH, we obtain a day 3 FSH concentration and consider a value less than 10 mIU/mL suggestive of adequate ovarian reserve, and levels of 10 to 15 mIU/mL borderline.

The upper threshold for a normal FSH concentration is laboratory dependent; cutoff values of 10 to 25 mIU/mL have been reported because of use of different FSH assay reference standards and assay methodologies.
DAY 3 ESTRADIOL

- There are conflicting data as to whether it is predictive of ovarian reserve and the response to ovarian stimulation (as in IVF).
- A value <80 pg/mL suggestive of adequate ovarian reserve, but other cut-offs are also utilized.
- In one prospective study of women undergoing IVF, day 3 estradiol levels >80 pg/mL resulted in higher cycle cancellation rates and lower pregnancy rates, and estradiol levels >100 pg/mL were associated with a 0 percent pregnancy rate.
DAY 3 ESTRADIOL

- Elevated basal estradiol levels are due to advanced premature follicle recruitment that occurs in women with poor ovarian reserve.
- High estradiol levels can inhibit pituitary FSH production and thus mask one of the signs of decreased ovarian reserve in perimenopausal women.
- Measurement of both FSH and estradiol levels helps to avoid false-negative FSH testing.
ANTI-MULLERIAN HORMONE

- AMH is produced by the granulosa cells of preantial and small antral follicles, beginning when primordial follicles start development and ending when they reach a diameter of 2-6 mm.

- The number of small antral follicles correlates with the size of the residual follicular pool and AMH levels decline progressively, becoming undetectable near the menopause.

- AMH can be measured anytime during the menstrual cycle.
ANTI-MULLERIAN HORMONE

- A serum AMH level above 0.5 ng/mL is consistent with good ovarian reserve. Levels less than 0.15 ng/mL suggest the patient will have a poor response to IVF.

- Low threshold values have good specificity for poor response to ovarian stimulation, but not for predicting pregnancy.
INHIBIN B

- Inhibin B is secreted primarily during the follicular phase by the granulosa cells of smaller antral follicles.
- Serum inhibin B concentrations increase in response to exogenous GnRH or FSH stimulation and vary widely across and between menstrual cycles.
- Inhibin B is generally not regarded as a reliable measure of ovarian reserve.
On transvaginal ultrasound, the presence of 4 to 10 antral follicles measuring between 2 and 10 mm in diameter suggests good ovarian reserve.
Assessment of fallopian tube patency
CHLAMYDIA ANTIBODIES

• Chlamydia trachomatis IgG antibody testing is a simple, inexpensive, noninvasive test with some evidence supporting its use as a method for predicting the presence of tubal disease.

• A negative test does not require further tubal assessment. If the test is positive, a sonohysterogram may be performed; presence of fluid in the cul-de-sac after intrauterine infusion of saline indicates patency of at least one tube.
CHLAMYDIA ANTIBODIES

- A cost-effective approach might be to screen women at low risk of tubal disease with chlamydia antibodies, followed by HSG if the test results are positive. Women at high risk of tubal disease would be screened by HSG primarily.

- Chlamydia antibody tests can provide useful information, but also have pitfalls that limit their clinical utility.
**CHLAMYDIA ANTIBODIES**

- It might be effective if limited to women with **unexplained infertility** (including a normal HSG), identifying those most likely to have undetected tubal factors best addressed before starting aggressive and costly empirical treatments.
- can be used to screen **women with an allergy to shellfish or iodinated contrast agents** who cannot undergo an HSG.
- The utility of chlamydia antibody tests in these or other clinical contexts is uncertain but warrants further investigation.
CERVICAL MUCUS

- accepts or captures sperm
- excludes the seminal plasma and morphologically abnormal sperm
- nurtures sperm biochemically
- serves as a reservoir
- prolonging sperm survival
- prolonging fertile interval between intercourse and ovulation.
CERVICAL MUCUS

- **Estrogen** stimulates cervical mucus production, and as levels rise during the follicular phase, mucus becomes more abundant and watery, less cellular, and more easily penetrated by sperm.

- **Progesterone** inhibits cervical mucus production and renders it opaque and impenetrable.
POST COITAL TEST

- The postcoital test is most frequently utilized to assess the adequacy of the cervical mucus and its interactions with sperm.

- After intercourse in the late follicular phase, the female partner is examined and a small amount of cervical mucus is obtained for assessment of spinnbarkeit (stretchability) and microscopic examination of ferning and sperm motility (at least 5 motile sperm per high power field is considered normal).
ABNORMAL POSTCOITAL TEST

- usually due to improper Timing.
- cervicitis
- Cryotherapy
- treatment with clomiphene citrate
- Absence of motile sperm in good quality mucus: ineffective intercourse, failed ejaculation (frequently resulting from performance anxiety), poor semen quality, and use of spermicidal coital lubricants.
ABNORMAL POSTCOITAL TEST

- A nucleic acid test for chlamydia and cultures for ureaplasma and mycoplasma
- Empirical treatment with antibiotics
- Semen analysis
Normal semen quality

absence of sperm in good quality mucus

1) Comparison of Partner and Donor sperm survival and motility in bovine cervical mucus
2) Anti sperm Ab
POST COITAL TEST

- There is no consensus on the normal range of sperm per high-power field and there is low inter- and intraobserver reproducibility.

- The marked heterogeneity of results and the limitations of study design raise serious doubts about the utility of the postcoital test.

- widely used infertility therapies (eg, intrauterine insemination, IVF) bypass the cervix.
KARYOTYPE

- In male partner if there is severe oligospermia (Separate testing for Y chromosome microdeletions may also be offered).
- Women with very early premature menopause (prior to age 40)
- In both partners if there have been recurrent pregnancy losses.
- In women with unexplained infertility, endometriosis, or tubal factor infertility (not in initial evaluation) Karyotype may be useful in patients with these conditions who have failed initial treatment approaches and plan to undergo IVF.
- Although the cost-effectiveness of universal karyotype screening prior to IVF has not been established.
TESTING FOR ANTIBODIES

- Routine testing for antiphospholipid, antisperm, antinuclear, and antithyroid antibodies is not supported by existing data.

- Although an association between antiphospholipid antibodies and recurrent pregnancy loss has been established, the other autoimmune factors remain under investigation as markers of fertility treatment failure.
POLYCYSTIC OVARIAN SYNDROME

Rotterdam criteria

• Oligo- and/or anovulation
• Clinical and/or biochemical signs of hyperandrogenism
• Polycystic ovaries (by ultrasound)
• In addition, other etiologies (congenital adrenal hyperplasias, androgen-secreting tumors, Cushing's syndrome) must be excluded
MENSTRUAL IRREGULARITY

- Biochemical evaluation of the oligomenorrhea is the same as in women with these disorders in the absence of PCOS.

- In addition to measurement of serum hCG to rule out pregnancy, minimal laboratory testing should include measurements of serum prolactin, TSH, and FSH to rule out hyperprolactinemia, thyroid disease, and ovarian failure respectively.
We do not routinely measure serum androgen concentrations in women with mild hirsutism.

In women with moderate to severe hirsutism, we typically measure a total and free testosterone concentration, and if there are concerns about a possible androgen-secreting tumor causing the hyperandrogenism, we add serum DHEA-S.

Other tests that should be considered depend upon the clinical presentation.
polycystic ovary syndrome
HYPERANDROGENISM

• An elevation in free testosterone is the most sensitive test to establish the presence of hyperandrogenemia. Elevated insulin and androgen levels both act to inhibit hepatic production of SHBG.

• Elevation of serum luteinizing hormone (LH) concentrations, normal serum estradiol, and increased serum estrone concentrations. None of these hormones are part of the diagnostic criteria for PCOS and, therefore, do not need to be measured.
Serum estrogen and gonadotropin concentrations in women with the polycystic ovary syndrome

![Graph showing hormone concentrations for LH, FSH, Estrone, and Estradiol in Day 2-4 cycle and PCOS.](image-url)
DYSLIPIDEMIA

- Lipid abnormalities, in particular low serum HDL, high serum triglycerides, and high serum LDL concentrations are common in women with PCOS.
- We suggest a fasting lipid profile in women with newly diagnosed PCOS.
OGTT

• Both lean and obese women with PCOS may have insulin resistance and hyperinsulinemia.

• Many obese women with PCOS are diagnosed with impaired glucose tolerance (up to 35 percent) or type 2 diabetes mellitus (up to 10 percent).

• A standard fasting glucose measurement lacks the sensitivity to detect impaired glucose tolerance or early type 2 diabetes.
Once the diagnosis of PCOS is made, we perform an OGTT in all patients.

When this is not practical, a FBS along with a hemoglobin A1C can be obtained.

If either one is abnormal, an OGTT should be performed to distinguish between IGT and diabetes.
Patients with normal glucose tolerance should be rescreened at least once every two years or more frequently if additional risk factors are identified.

Patients with impaired glucose tolerance should be screened annually for development of type 2 diabetes.
INSULIN RESISTANCE

- Hyperinsulinemic euglycemic clamp.
- Homeostatic model assessment of insulin resistance (HOMA-JR)
- Insulin sensitivity check index (QUICKI)
- The **fasting serum insulin** concentration is easy to obtain and requires no calculations; in euglycemic White women with PCOS, values greater than 20-30µU/mL suggest insulin resistance.
- The **fasting glucose/insulin ratio** has been used widely as an index of insulin sensitivity in women with PCOS; a ratio less than 4.5 has reasonable sensitivity and specificity for insulin resistance.
INSULIN RESISTANCE

- There currently is no validated test for measuring insulin resistance in a clinical setting.
- Using fasting insulin and glucose concentrations are sometimes used.
- Changes in beta-cell function over time, lack of a standardized universal insulin assay, and lack of data demonstrating that markers of insulin resistance predict response to treatment.
<table>
<thead>
<tr>
<th>Interpretation</th>
<th>2-hour Glucose</th>
<th>2-hour Insulin+</th>
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<tbody>
<tr>
<td>Normal</td>
<td>&lt;140 mg/dL</td>
<td></td>
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<tr>
<td>Impaired glucose tolerance</td>
<td>140–199 mg/dL</td>
<td></td>
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<tr>
<td>Diabetes mellitus</td>
<td>≥200 mg/dL</td>
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<tr>
<td>Normal</td>
<td>&lt;80–100 μU/mL</td>
<td></td>
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<tr>
<td>Insulin resistance</td>
<td>&gt;80–100 μU/mL</td>
<td></td>
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<tr>
<td>Severe insulin resistance</td>
<td>&gt;300 μU/mL</td>
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• As the sheer number of different measures of insulin resistance demonstrates, there is no uniformly accepted test for measuring insulin resistance in a clinical setting. Consequently, routine screening for insulin resistance is not recommended.
SCREENING RECOMMENDATION

- girls with premature adrenarche or menstrual irregularity that persists for more than 2 years after menarche because hyperinsulinemia often is the cause and they are at high risk for developing diabetes.

- for women with markely elevated serum androgen levels (≥150 ng/dL), to differentiate the severe insulin resistance syndromes from androgen-secreting tumors.
DIFFERENTIAL DIAGNOSIS

• Hyperprolactinemia

• Nonclassic CAH: A morning serum 17-hydroxyprogesterone ≥200 ng/dL (6 nmol/L) in the early follicular phase strongly suggests the diagnosis, which may be confirmed by a high dose (250 mcg) ACTH stimulation test. The diagnosis is excluded by a post-ACTH serum 17-hydroxyprogesterone value less than 1000 ng/dL (30 nmol/L).

• Androgen-secreting tumors: serum testosterone ≥ 150 ng/dL (5.2 nmol/L), and serum DHEAS ≥ 800 mcg/dL (21.6 µmol/L).
Thank you for your attention