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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Benign prostatic hyperplasia (BPH), also called **prostate enlargement**, is a noncancerous increase in size of the prostate gland. Symptoms may include frequent urination, trouble starting to urinate, weak stream, inability to urinate, or loss of bladder control. Complications can include urinary tract infections, bladder stones, and chronic kidney problems.

The cause is unclear. Risk factors include a family history, obesity, type 2 diabetes, not enough exercise, and erectile dysfunction. Medications like pseudoephedrine, anticholinergics, and calcium channel blockers may worsen symptoms. The underlying mechanism involves the prostate pressing on the urethra and thereby making it difficult to pass urine out of the bladder. Diagnosis is typically based on symptoms and examination after ruling out other possible causes.

About 105 million men are affected globally. BPH typically begins after the age of 40. Half of males age 50 and over are affected. After the age of 80, that figure climbs to as high as about 90% of males affected. Although prostate specific antigen levels may be elevated in males with BPH, the condition does not increase the risk of prostate cancer. The "**DISEASE DIAGNOSIS**" highlights BPH or BHP implying Benign prostatic hyperplasia for you.

"**INTERPRETATION**" segment outlines discretely how to understand AMH and how to interpret the results obtained.

"**TROUBLE SHOOTING**" identifies problems that can arise in Immunofluorescent assays and how to get rid of them.

Well, little fun and frolic has not been forgotten. Flip over please!

DISEASE DIAGNOSIS

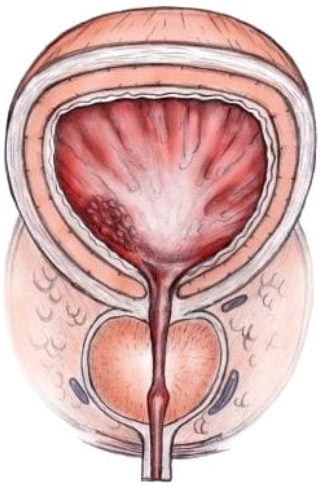
BENIGN HYPERPLASIA PROSTATE

Background

Benign prostatic hyperplasia (BPH), also known as benign prostatic hypertrophy, is a histologic diagnosis characterized by proliferation of the cellular elements of the prostate. Cellular accumulation and gland enlargement may result from epithelial and stromal proliferation, impaired preprogrammed cell death (apoptosis), or both. BPH involves the stromal and epithelial elements of the prostate arising in the periurethral and transition zones of the gland (see Pathophysiology). The hyperplasia presumably results in enlargement of the prostate that may restrict the flow of urine from the bladder. BPH is considered a normal part of the aging process in men and is hormonally dependent on testosterone and dihydrotestosterone (DHT) production. An estimated 50% of men demonstrate histopathologic BPH by age 60 years. This number increases to 90% by age 85 years. The voiding dysfunction that results from prostate gland enlargement and bladder outlet obstruction (BOO) is termed lower urinary tract symptoms (LUTS). It has also been commonly referred to as prostatism, although this term has decreased in popularity. These entities overlap; not all men with BPH have LUTS, and likewise, not all men with LUTS have BPH. Approximately half of men diagnosed with histopathologic BPH report moderate-to-severe LUTS. Clinical manifestations of LUTS include urinary frequency, urgency, nocturia (awakening at night to urinate), decreased or intermittent force of stream, or a sensation of incomplete emptying. Complications occur less commonly but may include acute urinary retention (AUR), impaired bladder emptying, the need for corrective surgery, renal failure, recurrent urinary tract infections, bladder stones, or gross hematuria. (See Presentation.) Prostate volume may increase over time in men with BPH. In addition, peak urinary flow, voided volume, and symptoms may worsen over time in men with untreated BPH (see Workup). The risk of AUR and the need for corrective surgery increases with age. Patients who are not bothered by their symptoms and are not experiencing complications of BPH should be managed with a strategy of watchful waiting. Patients with mild LUTS can be treated initially with medical therapy. Transurethral resection of the prostate (TURP) is considered the criterion standard for relieving bladder outlet obstruction (BOO) secondary to BPH. However, there is considerable interest in the development of minimally invasive therapies to accomplish the goal of TURP while avoiding its adverse effects (see Treatment).

Anatomy

The prostate is a walnut-sized gland that forms part of the male reproductive system. It is located anterior to the rectum and just distal to the urinary bladder. It is in continuum with the urinary tract and connects directly with the penile urethra. It is therefore a conduit between the bladder and the urethra. (See the image.)



Benign prostatic hyperplasia. Normal prostate anatomy is shown. The prostate is located at the apex of the bladder and surrounds the proximal urethra.

The gland is composed of several zones or lobes that are enclosed by an outer layer of tissue (capsule). These include the peripheral, central, anterior fibromuscular stroma, and transition zones. BPH originates in the transition zone, which surrounds the urethra.

Pathophysiology

Prostatic enlargement depends on the potent androgen dihydrotestosterone (DHT). In the prostate gland, type II 5-alpha-reductase metabolizes circulating testosterone into DHT, which works locally, not systemically. DHT binds to androgen receptors in the cell nuclei, potentially resulting in BPH. However, the fact that serum testosterone levels decrease with age, yet the development of BPH increases, suggests that other agents play an etiologic role. Possible factors include the metabolic syndrome, hyperinsulinemia, norepinephrine, angiotensin II, and insulin-like growth factors. In vitro studies have shown that large numbers of alpha-1-adrenergic receptors are located in the smooth muscle of the stroma and capsule of the prostate, as well as in the bladder neck. Stimulation of these receptors causes an increase in smooth-muscle tone, which can worsen LUTS. Conversely, blockade of these receptors (see Treatment) can reversibly relax these muscles, with subsequent relief of LUTS. Microscopically, BPH is characterized as a hyperplastic process. The hyperplasia results in enlargement of the prostate that may restrict the flow of urine from the bladder, resulting in clinical manifestations of BPH. The prostate enlarges with age in a hormonally dependent manner. Notably, castrated males (ie, who are unable to make testosterone) do not develop BPH. The traditional theory behind BPH is that, as the prostate enlarges, the surrounding capsule prevents it from radially expanding, potentially resulting in urethral compression. However, obstruction-induced bladder dysfunction contributes significantly to LUTS. The bladder wall becomes thickened, trabeculated, and irritable when it is forced to hypertrophy and increase its own contractile force. This increased sensitivity (detrusor overactivity), even with small volumes of urine in the bladder, is believed to contribute to urinary frequency and LUTS. The bladder may gradually weaken and lose the ability to empty completely, leading to increased residual urine volume and, possibly, acute or chronic urinary retention. In the bladder, obstruction leads to smooth-muscle-cell hypertrophy. Biopsy specimens of trabeculated bladders demonstrate evidence of scarce smooth-muscle fibers with an increase in collagen. The collagen fibers limit compliance, leading to higher bladder pressures upon filling. In addition, their presence limits shortening of adjacent smooth muscle cells, leading to impaired emptying and the development of residual urine. The main function of the prostate gland is to secrete an alkaline fluid that comprises approximately 70% of the seminal volume. The secretions produce lubrication and nutrition for the sperm. The alkaline fluid in the ejaculate results in liquefaction of the seminal plug and helps to neutralize the acidic vaginal environment. The prostatic urethra is a conduit for semen and prevents retrograde ejaculation (ie, ejaculation resulting in semen being forced backwards into the bladder) by closing off the bladder neck during sexual climax. Ejaculation involves a coordinated contraction of many different components, including the smooth muscles of the seminal vesicles, vasa deferentia, ejaculatory ducts, and the ischiocavernosus and bulbocavernosus muscles.

Epidemiology

BPH is a common problem that affects the quality of life in approximately one third of men older than 50 years. BPH is histologically evident in up to 90% of men by age 85 years. As many as 14 million men in the United

States have symptoms of BPH. Worldwide, approximately 30 million men have symptoms related to BPH.

Prognosis

In the past, chronic end-stage BOO often led to renal failure and uremia. Although this complication has become much less common, chronic BOO secondary to BPH may lead to urinary retention, chronic kidney disease, recurrent urinary tract infections, gross hematuria, and bladder calculi.

Patient Education

Patients should be informed that the following lifestyle changes may help relieve symptoms of BPH:

- Avoid alcohol and caffeine
- Avoid drinking fluids at bedtime; drink smaller amounts throughout the day
- Avoid taking decongestant and antihistamine medications
- Get regular exercise
- Make a habit of going to the bathroom when theyhave the urge
- Practice double voiding (empty the bladder, wait a moment, then try again)
- Practice stress management and relaxation techniques

Patients should be warned that if they become unable to urinate, they are at risk for permanent kidney or bladder injury and need to go to a hospital emergency department.

Clinical Presentation

History

The diagnosis of benign prostatic hyperplasia (BPH) can often be suggested on the basis of the history alone. Special attention to the following features is essential to making the correct diagnosis and recommending treatment choices:

- Onset and duration of symptoms
- General health issues (including sexual history)
- Fitness for any possible surgical interventions
- Severity of symptoms and how they are affecting quality of life
- Medications
- Previously attempted treatments

Symptoms often attributed to BPH can be caused by other disease processes, and a history and physical examination are essential in ruling out other etiologies of (lower urinary tract symptoms (LUTS).

When the prostate enlarges, it may act like a "clamp on a hose," constricting the flow of urine. Nerves within the prostate and bladder may also play a role in causing the following common symptoms:

- Urinary frequency - The need to urinate frequently during the day or night (nocturia), usually voiding only small amounts of urine with each episode
- Urinary urgency - The sudden, urgent need to urinate, owing to the sensation of imminent loss of urine without control
- Hesitancy - Difficulty initiating the urinary stream; interrupted, weak stream
- Incomplete bladder emptying - The feeling of persistent residual urine, regardless of the frequency of urination
- Straining - The need strain or push (Valsalva maneuver) to initiate and maintain urination in order to more fully evacuate the bladder
- Decreased force of stream - The subjective loss of force of the urinary stream over time
- Dribbling - The loss of small amounts of urine due to a poor urinary stream

A sexual history is important, as epidemiologic studies have identified LUTS as an independent risk factor for erectile dysfunction and ejaculatory dysfunction.

Physical Examination

Conduct a focused physical examination to assess the suprapubic area for signs of bladder distention and a neurological examination for sensory and motor deficits. The digital rectal examination (DRE) is an integral part of the evaluation in men with presumed BPH. During this portion of the examination, prostate size and contour can be assessed, nodules can be evaluated, and areas suggestive of malignancy can be detected. Decreased anal sphincter tone or the lack of a bulbocavernosus muscle reflex may indicate an underlying neurological disorder. The prostate is examined using the index finger of the dominant hand. The finger is placed through the anus after relaxation of the anal sphincter, and the prostate is palpated circumferentially (analogous to a windshield wiper movement). In general, an estimation of the number of index finger pads that one can sweep over the rectal surface of the prostate during DRE is a useful way for non-urologist examiners to communicate estimated gland size. For example, one can report the prostate size as "2-3 fingerbreadths wide" when charting in the medical record or communicating with a colleague. Most asymptomatic men have glands of 2 fingerbreadths or less. Anecdotally, each fingerbreadth correlates to approximately 15-20 g of tissue. The normal prostate volume in a young man is approximately 20 g. In addition, pelvic floor tone, the presence or absence of fluctuance (ie, prostate abscess), and pain sensitivity of the gland (prostatodynia/prostatitis) can be assessed. A more precise volumetric determination can be made using transrectal ultrasonography (TRUS) of the prostate.

Complications

Complications related to bladder outlet obstruction (BOO) secondary to BPH include the following:

- Urinary retention
- Renal insufficiency
- Recurrent urinary tract infections
- Gross hematuria
- Bladder calculi
- Renal failure or uremia (rare in current practice)

Differential Diagnoses

Symptoms often attributed to benign prostatic hyperplasia (BPH) can be caused by any of the following conditions:

- Cystitis
- Prostatitis
- Prostatodynia
- Prostatic abscess
- Overactive bladder (OAB)
- Carcinoma of the bladder
- Foreign bodies in the bladder (stones or retained stents)
- Urethral stricture due to trauma or a sexually transmitted disease
- Prostate cancer
- Neurogenic bladder
- Pelvic floor dysfunction

Excluding these entities based on findings from a thorough history and appropriately directed diagnostic studies is essential.

Differential Diagnoses

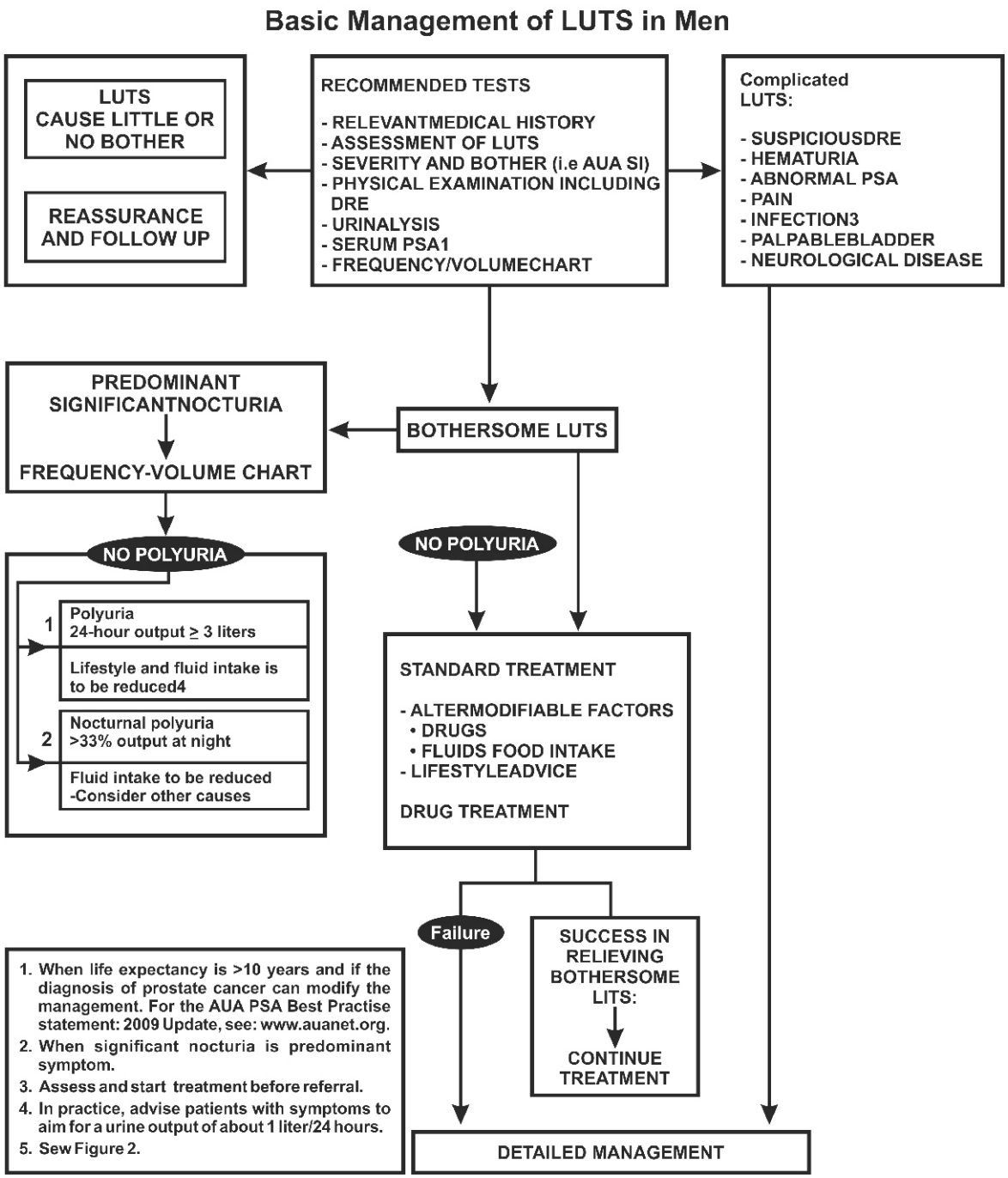
- Bladder Cancer
- Bladder Stones
- Bladder Trauma
- Interstitial Cystitis

- Neurogenic Bladder
- Prostatitis
- Radiation Cystitis
- Urethral Strictures in Males
- Urinary Tract Infection (UTI) in Males

Workup

Approach Considerations

The guideline includes an algorithm for the diagnosis and basic treatment of lower urinary tract symptoms (LUTS), which is presented below.



Benign prostatic hyperplasia. Basic management of lower urinary tract symptoms (LUTS) in men.

The Diagnosis Improvement in PrimAry Care Trial (D-IMPACT), a prospective, multicenter study in three European countries, identified simple tests for primary care practitioners to diagnose BPH in men who present with LUTS. D-IMPACT found that a diagnostic algorithm including only the objective variables of age, International Prostate Symptom Score (IPSS) and prostate-specific antigen level (PSA), allows accurate diagnosis of BPH in approximately three-quarters of patients who report LUTS.

Urinalysis and Urine Culture

Examine the urine using dipstick methods and/or via centrifuged sediment evaluation to assess for the presence of blood, leukocytes, bacteria, protein, or glucose. A urine culture may be useful to exclude infectious causes of irritative voiding. It is usually performed if the initial urinalysis findings indicate an abnormality.

Prostate-Specific Antigen

Although BPH does not cause prostate cancer, men at risk for BPH are also at risk for prostate cancer and should be screened accordingly. Screening for prostate cancer remains controversial and should done after an informed discussion between the physician and patient. The current American Cancer Society (ACS) guideline for early detection of prostate cancer stresses the importance of involving men in the decision whether to test for prostate cancer. The ACS notes that PSA testing may reduce the likelihood of dying from prostate cancer but poses serious risks, particularly of treatment of prostate cancer that would not have caused ill effects if left undetected. The ACS recommends that men receive information about the uncertainties, risks, and potential benefits associated with prostate cancer screening. After this discussion, if the patient wishes to proceed with screening (ie, prostate-specific antigen [PSA] testing and digital rectal examination [DRE] for prostate cancer), the ACS recommends that screening start at the following ages:

- Age 50 years in men at average risk for prostate cancer who are expected to live at least 10 more years
- Age 45 years in men at high risk for prostate cancer (African Americans and men with a first-degree relative diagnosed with prostate cancer before age 65)
- Age 40 years in men at very high risk (those with more than one first-degree relative who had prostate cancer at an early age).

A physician should discuss the risks and benefits of PSA screening with the patient. Notably, men with larger prostates may have slightly higher PSA levels.

Electrolytes, BUN, and Creatinine

These evaluations are useful screening tools for chronic renal insufficiency in patients who have high post-void residual (PVR) urine volumes. A routine serum creatinine measurement is not indicated in the initial evaluation of men with lower urinary tract symptoms (LUTS) secondary to BPH.

Ultrasonography

Ultrasonography (abdominal, renal, transrectal) and intravenous urography are useful for helping determine bladder and prostate size and the degree of hydronephrosis (if any) in patients with urinary retention or signs of renal insufficiency. Generally, they are not indicated for the initial evaluation of uncomplicated LUTS. A systematic review concluded that patients with suspected large post-void residual volumes should undergo a bladder scan for urine volume to assess for bladder outlet obstruction. Urine volumes measured by bladder scanning correlated highly with urine volumes measured by bladder catheterization. Symptoms alone proved insufficient for diagnosis, although an International Prostate Symptom Score of 20 or greater increased the likelihood of bladder outlet obstruction. Transrectal ultrasonography (TRUS) of the prostate is recommended in selected patients, to determine the dimensions and volume of the prostate gland. The success of certain minimally invasive treatments may depend on the anatomical characteristics of the gland. In patients with elevated PSA levels, TRUS-guided biopsy may be indicated to assess for prostate cancer. Imaging of the upper tracts is indicated in patients who present with any of the following:

- Concomitant hematuria
- A history of urolithiasis
- An elevated creatinine level
- High PVR volume
- History of upper urinary tract infection

Other imaging studies, such as CT scanning and MRI, have no role in the evaluation and treatment of uncomplicated BPH.

Endoscopy of the Lower Urinary Tract

Cystoscopy may be indicated in patients scheduled for invasive treatment or in whom a foreign body or malignancy is suspected. In addition, endoscopy may be indicated in patients with a history of sexually transmitted disease (eg, gonococcal urethritis), prolonged catheterization, or trauma. Findings may suggest urethral stricture as the cause of BOO, instead of BPH. Flexible cystoscopy can be easily performed in several minutes in an office-based setting using topical gel-based intraurethral anesthesia without sedation. The appearance of the gland alone on cystoscopy cannot make the diagnosis of obstruction but can help the clinician decide on treatment modalities if intervention is warranted.

Histologic Findings

BPH is characterized by a varying combination of epithelial and stromal hyperplasia in the prostate. Some cases demonstrate an almost pure smooth-muscle proliferation, although most demonstrate a fibroadenomyomatous pattern of hyperplasia. In the bladder, obstruction leads to smooth-muscle-cell hypertrophy. Biopsy specimens of trabeculated bladders demonstrate evidence of scarce smooth-muscle fibers with an increase in collagen.

Treatment & Management

Pharmacologic treatment

Agents used in the treatment of BPH include the following:

- Alpha-adrenergic receptor blockers
- 5-alpha reductase inhibitors
- Phosphodiesterase-5 enzyme inhibitors
- Anticholinergic agents

Surgery

- Transurethral resection of the prostate (TURP) - The criterion standard for relieving BOO secondary to BPH
- Open prostatectomy - Reserved for patients with very large prostates (>75 g), patients with concomitant bladder stones or bladder diverticula, and patients who cannot be positioned for transurethral surgery

Minimally invasive treatment

- Transurethral incision of the prostate (TUIP)
- Laser treatment - Used to cut or destroy prostate tissue; multiple laser types are available, including green light, holmium, and thulium, and each has its own strengths and weaknesses
- Transurethral microwave therapy (TUMT) - Generates heat that causes cell death in the prostate, leading to prostatic contraction and volume reduction
- Transurethral needle ablation of the prostate (TUNA)
- High-intensity ultrasonographic energy therapy - Currently in the clinical trial stage
- Prostatic stents - Flexible devices that expand when put in place to improve the flow of urine past the prostate
- Laparoscopic prostatectomy
- Implanted devices to relieve prostatic obstruction (eg, UroLift)
- Prostate artery embolization - Performed by a radiologist; this technique has yet to become established as a standard-of-care therapeutic option.

INTERPRETATION

Anti-Müllerian Hormone Test

What is an anti-müllerian hormone (AMH) test?

This test measures the level of anti-müllerian hormone (AMH) in the blood. AMH is made in the reproductive tissues of both males and females. The role of AMH and whether levels are normal depend on your age and gender. AMH plays an important role in the development of sex organs in an unborn baby. During the first weeks of pregnancy, a baby will start developing reproductive organs. The baby will already have the genes to become either a male (XY genes) or a female (XX genes). If the baby has male (XY) genes, high levels of AMH are made, along with other male hormones. This prevents the development of female organs and promotes the formation of male organs. If there is not enough AMH to stop the development of female organs, organs of both sexes may form. When this happens, a baby's genitals may not be clearly identified as male or female. This is known as ambiguous genitalia. Another name for this condition is intersex. If the unborn baby has female (XX) genes small amounts of AMH are made. This allows for the development of female reproductive organs. AMH has a different role for females after puberty. At that time, the ovaries (glands that make egg cells) begin making AMH. The more egg cells there are, the higher the level of AMH. In women, AMH levels can provide information about fertility, the ability to get pregnant. The test may also be used to help diagnose menstrual disorders or to monitor the health of women with certain types of ovarian cancer. Other names: AMH hormone test, müllerian-inhibiting hormone, MIH, müllerian inhibiting factor, MIF, müllerian-inhibiting substance, MIS.

What is it used for?

An AMH test is often used to check a woman's ability to produce eggs that can be fertilized for pregnancy. A woman's ovaries can make thousands of eggs during her childbearing years. The number declines as a woman gets older. AMH levels help show how many potential egg cells a woman has left. This is known as the ovarian reserve. If a woman's ovarian reserve is high, she may have a better chance of getting pregnant. She may also be able to wait months or years before trying to get pregnant. If the ovarian reserve is low, it may mean a woman will have trouble getting pregnant, and should not delay very long before trying to have a baby.

AMH tests may also be used to:

- Predict the start of menopause, a time in a woman's life when her menstrual periods have stopped and she can't become pregnant anymore. It usually starts when a woman is around 50 years old.
- Find out the reason for early menopause.
- Help find out the reason for amenorrhea, the lack of menstruation. It is most often diagnosed in girls who haven't started menstruating by the age of 15 and in women who have missed several periods.
- Help diagnose polycystic ovary syndrome (PCOS), a hormonal disorder that is a common cause of female infertility, the inability to get pregnant.
- Check infants with genitals that are not clearly identified as male or female.
- Monitor women who have certain types of ovarian cancer.

Why do I need an AMH test?

You may need an AMH test if you are a woman who is having difficulty getting pregnant. The test can help show what your chances are of conceiving a baby. If you are already seeing a fertility specialist, your doctor may use the test to predict whether you will respond well to treatment, such as in vitro fertilization (IVF). High levels may mean you may have more eggs available and will respond better to treatment. Low levels of AMH mean you may have fewer eggs available and may not respond well to treatment.

You may also need an AMH test if you are a woman with symptoms of polycystic ovary syndrome (PCOS). These include:

- Menstrual disorders, including early menopause or amenorrhea
- Acne
- Excess body and facial hair growth
- Decreased breast size
- Weight gain

In addition, you may need an AMH test if you are being treated for ovarian cancer. The test can help show if your treatment is working.

What do the results mean?

If you are a woman trying to get pregnant, your results can help show what your chances are for conceiving. It can also help you decide when to try to get pregnant. A high level of AMH can mean your chances are better and you may have more time before trying to get pregnant. A high level of AMH may also mean you have polycystic ovary syndrome (PCOS). There is no cure for PCOS, but symptoms can be managed with medications and/or lifestyle changes, such as maintaining a healthy diet and waxing or shaving to remove excess body hair. A low level can mean you may have trouble getting pregnant. It can also mean that you are starting menopause. A low level of AMH is normal in young girls and in women after menopause. If you are being treated for ovarian cancer, your test can show whether your treatment is working. In a male infant, a low level of AMH may mean a genetic and/or hormonal problem causing genitals that are not clearly male or female. If AMH levels are normal, it may mean the baby has working testicles, but they are not in the right location. This condition can be treated with surgery and/or hormone therapy. If you have questions about your results, talk to your health care provider. Learn more about laboratory tests, reference ranges, and understanding results.

Is there anything else I need to know about an AMH test?

If you are a woman being treated for fertility problems, you will probably get other tests, along with AMH. These include tests for estradiol and FSH, two hormones involved in reproduction.

AMH VALUES

- **Average:** Between 1.0 ng/mL to 3.0 ng/mL.
- **Low:** Under 1.0 ng/mL.
- **Severely low:** 0.4 ng/mL.

TROUBLESHOOTING

IMMUNOFLUORESCENCE TROUBLESHOOTING

Identify the problem with your immunofluorescence staining from the options below:

Weak or No Staining
High Background
Non-specific Staining

WEAK OR NO STAINING

Incorrect light source/filter set:
- Ensure your microscope is equipped with the correct light source and filter set for the fluorophore you have chosen.

Gain/exposure is too low:
- Turn up the gain and/or increase the exposure time to ensure you are capturing any signal present.

Fluorescent tag bleached:
- Avoid over exposure of the slide to light sources for extended periods. Always store slides in the dark.

Cell/tissues are over fixed:
- Reduced the duration of fixation.
- Perform antigen retrieval to unmask the epitope.

Cells were not permeabilized:
- Methanol and acetone fixation will permeabilize cells.
- If using formaldehyde, permeabilize cells with 0.2% Triton X-100.

Tissue/cells dried out:
- Samples must be kept covered in liquid throughout the staining process.

Not enough primary antibody:
- Use a higher concentration of antibody.
- Incubate longer.

The primary and secondary antibodies are incompatible:
- The secondary antibody should be raised against the host of the primary antibody. For example, if the primary antibody is a Mouse Anti-HSP70, use an Anti-Mouse secondary antibody (ie. Goat Anti-Mouse).
- Isotypes should also be compatible.

Suitability of the primary antibody:
- Confirm that the antibody has been validated in IHC, and specifically what type- formalin fixed, paraffin-embedded, fresh frozen, etc.
- Test the antibody in a western blot to make sure it hasn't been damaged.

Slide storage issues:
- Samples should be imaged shortly after processing as the signal decreases over time. Store slides at 4°C in the dark if needed.

Antibody Storage issues:
- Freeze/thaw cycles are detrimental and can cause degradation. It is best to create aliquots of smaller amounts as soon as the product arrives at your location.
- Antibody was not stored as recommended. Unfortunately this might require a new vial to be used instead.
- If the secondary was not stored in the dark (when using immunofluorescence), a new vial will need to be used instead.

The protein is not present in the tissues being tested:
- Run a positive control.
- If the protein is present, but not abundantly, use an amplification step to maximize the signal.

Incubation time is too short:
- Increase the duration of incubation of the primary antibody with the sample.

HIGH BACKGROUND

Autofluorescence:
- Check to see if there is any fluorescence in an unstained section of the processed tissue. If there is, then this is autofluorescence in the tissue.
- Avoid glutaraldehyde fixative or wash with 0.1% sodium borohydride in PBS to remove free aldehyde groups.
- May be due to endogenous molecules (FAD, FMN, NADH, lipofuscin), and will require pre-photobleaching, treatment with sudan black, or cupric sulfate.

Tissue is too thick:
- Consider using thinner tissue sections.

Antibody Concentration is too high:
- Reduce the concentration of the primary and/or secondary antibody used.

Secondary is binding non-specifically:
- Run a secondary control without the primary. If there is staining, then change the secondary.

Blocking is insufficient:
- Increase the blocking incubation period and consider changing the blocking agent.

Amplification:
- Reduce amplification incubation time and dilute the secondary antibody.

Insufficient washing:
- Proper washing of the tissue between steps is critical. Ensure you are following the protocol guidelines for wash steps.

NON-SPECIFIC STAINING

Spectral overlap:
- If imaging more than one fluorescent probe, the fluorophores may have excitation and emission spectra that overlap. Adjust your light sources and filters to pick up only one signal at a time. If this is not possible, choose new fluorophores that do not have spectral overlap.

Antibody Concentrations too high:
- Try reducing the concentration, and the incubation period.

The primary is raised again the same species as the tissues stained (eg. Mouse on mouse):
- Try using a primary that is raised against a different species. Otherwise try to block the endogenous IgG with serum from the same species as the secondary. You can also try to incubate sections with 1% Triton to clean the tissues. Or Use TBS-Tween 20 as a washing buffer, rather than using PBS-Tween 20.

Aggregates:
- Spin down secondary antibodies in a microcentrifuge to move aggregates to the bottom of the tube. Take from the top.

BOUQUET

Wisdom Whispers

In Lighter Vein

1st son : Degree in Economics.
2nd son: MBA.
3rd son : PhD
4th son : Thief

Neighbour: Why can't you throw the 4th son out of your house?

Father : He is the only one earning money. The rest are unemployed.

Wife : had ur lunch.?
Husband : had ur lunch.?
Wife : i m asking you
Husband : i m asking you
Wife : u copying me.?
Husband : u copying me?
Wife : lets go shopping
Husband :Yes i had my lunch

Customer : Waiter, what kind of a drink is this? I ordered guava juice but this tastes like kerosene.

Waiter : Sorry Sir, that must be apple juice. Our guava juice here tastes like soap.

Brain Teasers

1. **Which of the following can demonstrate PSA**
   A. Breast milk (human)
   B. Breast Nipple aspirate.
   C. None of the above
   D. Both of the above

2. **In relation to Prostate. What does I stand for in PIN?**
   A. New
   B. Natural
   C. Neoplasia
   D. Necrosis

3. **What is the normal value of AMH in women?**
   A. 1 to 3 NG/ML
   B. 3 to 5 NG/ML
   C. 5 to 10 NG/ML
   D. 10-25 NG/ML

4. **In relation to Cancer, what technique do Pathologists/ Molecular Pathologists use?**
   A. DISH
   B. FISH
   C. MOUSE
   D. GOAT

ANSWER: 1: D, 2: C, 3: A, 4: B

FIAcheck™ Towards The future of Fluorescence

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FIAcheck™ 1.0

Time Resolved Fluorescence Analyzer

FIAcheck™ Parameter Menu

Thyroid Markers	TSH	25T
	TT3	25T
	TT4	25T
Fertility Marker	FSH	25T
	LH	25T
	PRL	25T
	β-hCG	25T
	AMH	10T
Cancer Marker	PSA	10T
Rhematology	Anti-CCP	10T

Diabetic Marker	HbA1C	25T
Inflamation	CRP	25T
	PCT	10T
	IL-6	10T
Anemic Marker	Ferritin	10T
Coagulation	D-Dimer	10T
Cardiac Markers	cTnI	10T
	NT-proBNP	10T
Vitamins	Vitamin D	10T
	Vitamin B12	10T
Allergy	IgE	10T

Not all Fluorescence Analyzers are time resolved

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