Second Level
Male Infertility Assessment

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Endocrine Evaluation

- Spermatogenesis: highly dependent on intratesticular testosterone synthesis
- Testosterone levels→ vary widely: 280-300 ng/dL as a threshold for adequate androgenization
- sperm density of ≤22 million/mL: will not have conceived with their partners within 1 year → one third related to endocrinopathy

- Laboratory evaluation of Androgenization: in all men presenting for infertility (including those in whom sperm density is greater than 20 million/mL)
- Testosterone: three main forms
- 1) tightly bound to SHBG(30-44%)
- 2) loosely bound to protein, primarily albumin(54-68%)
- 3) Unbound or free(0.5-3.0%)
- The forms inducing cellular activity: free and loosely bound→ *bioavailable testosterone*

- Bioavailable testosterone: to calculate it from total testosterone, SHBG, and albumin
- Circadian rhythm of total serum testosterone: a peak in the early morning and trough levels in the late afternoon
- SHBG displays an opposing circadian rhythm in men of all ages
- In older men→ total testosterone + bioavailable testosterone and circadian rhythm are attenuated
- Assays are typically performed in the morning, especially in younger men

- Hypoandrogenism, a pituitary or testicular source→ assessing LH
- Testicular Leydig cell dysfunction→ ↑LH
- Pituitary dysfunction→ ↓LH
- testosterone and LH: released in a pulsatile fashion→ borderline results may be investigated further by 3 morning samples at 20-minute intervals

- The Sertoli cell products regulate pituitary FSH by:
- Inhibiting its release→ inhibin B
- Stimulating its release→ activin

- Sertoli cells are regulated by robust paracrine interaction with germ cells
- Depopulation of germ cells→ ↓inhibin → ↑FSH
- FSH: an indirect assessment of germ cell mass and its function

- Testis size + FSH→ an accurate predictor
- 96% of men with OA: FSH≤7.6 IU/L+ testis long axis≥4.6 cm
- 89% of men with NOA(spermatogenic dysfunction): FSH≥7.6 IU/L + testis long axis≤4.6 cm

- Assays of inhibin B: many studies observe greater accuracy with measuring inhibin B than with FSH
- Lower ranges of inhibin B allow improved correlation
- Incremental improvement in accuracy: is small + cost and availability

- AMH: a member of TGF-β family→ synthesized by Sertoli cells
- Results from pilot studies: encouraging, but small→ primarily experimental

- Aromatase enzymes→ convert testosterone to estrogens
- ↑Estradiol adversely affects male reproductive potential
- A ratio of total testosterone to estradiol < 10:1→ indicate reproductive dysfunction

- Prolactin: inhibit gonadotropins → ↓testosterone
- ↑PRL:in pituitary hyperplasia, adenoma, or tumors
- Clinically significant disease of the pituitary→ symptoms such as visual field changes, headache, or erectile dysfunction
- Prolactin assay: these symptoms + male infertility; Especially if testosterone is low
- Very low incidence + labile assay +Repetition of the test

- Assessment of other pituitary hormones: if a space-occupying pituitary lesion is suspected or found on imaging examination

- A reasonable initial laboratory screen→ performed in the **morning** + includes:
- **total testosterone** + **SHBG** + **Albumin**
- **LH** and **FSH**
- **estradiol**

Genomic Assessment

- **Karyotype:**
- 1) all males with azoospermia caused by spermatogenic dysfunction
- 2) severe oligospermia ≤5 million sperm/mL

- vary by geographic region + significant expense → treating physician may judge whether this assay is indicated in his or her patient population

- **Y Chromosome Microdeletion Testing:**
- Determinant of the male gender
- Passed directly from father to son
- A region in the long arm of the Y chromosome: critical to the formation of sperm in man→ ***AZF*** *(azoospermia factor)*
- Microdeletions of three regions on the Y chromosome: commonly associated with azoospermia or oligospermia→ termed *AZFa, AZFb,* and *AZFc*

- The *DAZ* genes within the AZFc region: integrally associated with spermatogenesis
- Proximal portion of AZFc: *AZFd*→ unclear
- Some microdeletions of AZFc→ spermatogenic impairment but not failure
- AZFa and AZFb microdeletions cause significant pathology of the testis resulting in diminishing low likelihood of sperm retrieval by surgery

- Recommend Y chromosomal microdeletion assessment→ azoospermic men before surgical sperm extraction (to counsel them on the likelihood of retrieval)
- Also reasonable to omit testing→ the relative rarity of AZFa and AZFb microdeletions in clinical practice

- **Genomic Sequence Assessment:**
- **DNA microarrays**: allow multiple single nucleotide polymorphisms (SNPs) and mutations associated with known diseases to be screened
- Identify whether parents are carriers for a large number of genetic diseases and the probability of affected offspring
- **Whole genome sequencing**: a clinical tool→ under current development
- As general screening tools in evaluating male infertility is not yet warranted

- **Cystic Fibrosis Transmembrane Conductance Regulator Mutation Assessment:**
- Stratified by ethnicity
- Maldevelopment of the vas

Secondary Semen Assays

- **Antisperm Antibody:**
- Direct assays: on the surface of sperm→ preferred
- 1) the mixed antiglobulin reaction (MAR) test
- 2) the immunobead assay

- Indirect assays: in fluid such as seminal plasma or serum

- **Pyospermia Assays:** threshold =1 million/mL
- presence of immature germ cells is common and not of pathologic significance

Tertiary and Investigational Sperm Assays

- **Sperm DNA Integrity Assays**: fragmentation or disturbances in DNA arrangement→ aberrations in sperm function, fertilization, implantation, and pregnancy
- Two types of test methods:
- 1) directly measure DNA fragmentation→ more effectively correlate with clinical outcomes
- 2) In the other→ DNA is denatured before analysis

- ↑DFI: double risk of miscarriage

- **TUNEL** Assay
- A direct measure
- Risk ratio of 4 in miscarriage rates

- **Comet** Assay
- At neutral pH→ a direct measure
- To understand the effects of various entities on sperm DNA, including varicocele, toxins, male age, and testis cancer

- **Denatured Sperm DNA Assays**
- Comet assay in acidic or alkaline conditions
- Sperm chromatin dispersion (SCD) assay
- Allows visual identification of individual sperm head DNA structure

- The most established assay for sperm head DNA structure: Sperm Chromatic Structure Assay (SCSA)
- Does not identify individual sperm
- But rather a population of cells
- In miscarriage rates: SCSA had a RR of 1.47→ a weak likely association

- **Reactive Oxygen Species**
- **ROS**s *are involved in multiple* physiologic processes
- If present in excess: seminal ROSs may cause reproductive dysfunction (
- TAC in seminal fluid
- ROS-TAC score
- ROS activity in aging, prostatitis, varicocele, lubricants, radiation, smoking, toxins, and obesity

- Acrosome Reaction
- Sperm Mucous Interaction
- Sperm Ovum Interaction
- Sperm Ultrastructural Assessment