Abnormalities of Spermatogenesis
Male Factor

40% of the cause for infertility

Sperm is constantly produced by the germinal epithelium of the testicle

Sperm generation time 73 days

Sperm production is thermoregulated

1°F less than body temperature

Both men and women can produce anti-sperm antibodies which interfere with the penetration of the cervical mucus
Semen Analysis (SA)

Obtained by masturbation
Provides immediate information
Quantity
Quality
Density of the sperm
Abstain from coitus 2 to 3 days
Collect all the ejaculate
Analyze within 1 hour
A normal semen analysis excludes male factor 90% of the time
Morphology
Motility
# Normal Values for SA

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Value</th>
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<tbody>
<tr>
<td>Volume</td>
<td>2.0 ml or more</td>
</tr>
<tr>
<td>Sperm Concentration</td>
<td>20 million/ml or more</td>
</tr>
<tr>
<td>Motility</td>
<td>50% forward progression</td>
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<tr>
<td>Viscosity</td>
<td>25% rapid progression</td>
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<tr>
<td>Morphology</td>
<td>Liquification in 30-60 min</td>
</tr>
<tr>
<td>pH</td>
<td>30% or more normal forms</td>
</tr>
<tr>
<td>WBC</td>
<td>7.2-7.8</td>
</tr>
<tr>
<td></td>
<td>Fewer than 1 million/ml</td>
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</table>
Causes for male infertility

42% varicocele
repair if there is a low count or decreased motility

22% idiopathic

14% obstruction

20% other (genetic abnormalities)
Abnormal Semen Analysis

Azospermia
Klinefelter’s (1 in 500)
Hypogonadotrophic-hypogonadism
Ductal obstruction (absence of the Vas deferens)

Oligospermia
Anatomic defects
Endocrinopathies
Genetic factors
Exogenous (e.g. heat)
Abnormal volume
Retrograde ejaculation
Infection
Ejaculatory failure
Evaluation of Abnormal SA

Repeat semen analysis in 30 days
Physical examination
Testicular size
Varicocele
Laboratory tests
Testosterone level
FSH (spermatogenesis- Sertoli cells)
LH (testosterone- Leydig cells)
Referral to urology
SEmen Analysis

Dr. Amal Baalash
Indications

- Assessment of fertility
- Forensic purposes
- Effectiveness of vasectomy - 2 samples 1 month apart negative
- Suitability for artificial insemination
Semen Analysis Include

- Macroscopic
  - viscosity
  - coagulation + liquifaction
  - volume
  - pH

- Microscopic
  - concentration/count
  - motility
  - morphology
  - viability

Motility & Viability must be performed within 1½ - 2 hrs of collection
REMEMBER
SEMEN IS A BODY FLUID
BIOHAZARDOUS
Semen Collection

- Name
- Period of abstinence - 2-7 days
- Time of collection
- Entire ejaculate and not coitus interruptus in a wide mouth container
- Delivered within 1 hour of collection
- Avoid temperature extremes
Reference Ranges

- Volume  2.0-6.0 ml
- pH 7.2-8.0
- Count >20 million/ml
- Total count > 40 million
- Morphology > 30% normal form
- Viability > 75% (50% in other)
- WBC< 1million/ml
- RBC none
Macroscopic Examination

- Semen is viscous, yellow grayish.
- Forms gel-like clot immediately.
- Liquefies completely in 5-60 minutes; this must be complete before further testing (mix before further testing).
- Appearance: homogenous white-gray opalescence.
  - Brown/red in hematospermia
  - Dense white turbid if inflammation and high WBC
Macroscopic Examination

- **Volume:** in graduated cylinder to the nearest 0.1 ml or centrifuge tube free of contamination.
- **Viscosity:** 5ml pipette or plastic pipette
  - normal, more viscous, very viscous
- **pH:** important parameter of motility and viability 7.2-8.0; measured by pH paper.
Motility

- While estimating count
- No stain
- Count 200 total sperm and then the motile
- Calculate the percentage of
  - Progressive motile
  - Sluggishly motile (<5 um/s)
  - nonmotile
- >50% motile
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<table>
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<tbody>
<tr>
<td>0</td>
<td>No movement</td>
</tr>
<tr>
<td>1</td>
<td>Movement, none forward</td>
</tr>
<tr>
<td>1+</td>
<td>Occasional movement of a few sperm</td>
</tr>
<tr>
<td>2</td>
<td>Slow, undirected</td>
</tr>
<tr>
<td>2+</td>
<td>Slow, directly forward movement</td>
</tr>
<tr>
<td>3−</td>
<td>Fast, but undirected movement</td>
</tr>
<tr>
<td>3</td>
<td>Fast, directed forward movement</td>
</tr>
<tr>
<td>3+</td>
<td>Very fast forward movement</td>
</tr>
<tr>
<td>4</td>
<td>Extremely fast forward movement</td>
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</table>
Agglutination

- Reported when motile sperm stick to each other in a definite pattern.
  - Head-head
  - Tail-tail
  - Head-tail
- Immunological cause of infertility
- Done on several HPF
Viability

- Supravital stain:
  - Eosin +/- Nigrosin
    - Viable do not take up the stain
- This distinguish live nonmotile from dead; it is important to compare viability and motility.
Morphology

- Smear:
  - H&E, Papanicolaou, Wright stains
  - Feathering like blood smear or 2 slides
  - Count and classify 100-200 spermatozoa
  - Examine the head, midpiece, tail
- Normal >30%
- Immature
- Abnormal
Mira1000 Semen Analyzer (CASA)
Aspermia: No semen ejaculated
Hematospermia: Blood present in semen
Leucocytospermia: White blood cells present in semen
Azospermia: No spermatozoa found in semen
Normospermia: Normal semen parameters
Oligospermia: Low sperm concentration
Asthenospermia: Poor motility and/or forward progression
Teratospermia: Reduced percentage of morphologically normal sperm
Necrospermia: No live sperm in semen
Other Sperm Abnormalities

- Head abnormalities:
  - absence
  - double head
  - micro/megalo

- Tail abnormalities:
  - coiled
  - kinked
  - lengthened
Sperm Count

- Manual methods
  - Hemocytometer or counting chamber
- Computer assisted
- Oligospermia < 20 million
- If azospermia: fructose level must be ordered to verify the integrity of the vas and seminal vesicles
Preparation

- Manual methods
  - Hemocytometer or counting chamber
- Computer assisted

1. Thoroughly mix specimen and dilute 1:10 with diluent. (To obtain this dilution, dilute 100 uL of liquefied semen with 900 uL of diluent)

2. Thoroughly mix diluted specimen and allow a drop (10 - 20 uL) to into each side of the hemocytometer covered with a coverglass.

3. Allow chamber to stand for about 5 minutes in a humid container to prevent drying. During this period, the cells settle and can be more easily counted.
4. After cells have settled, place chamber under a microscope.
5. Count spermatozoa present in 5 1/25mm squares in center square millimeter X5 or sperms in one of the 9 large squares. Only morphologically mature germinal cells with tails are counted.

No of sperms per large square X dilution factor (10) X Depth of chamber (10) X 1000 = count in million/ ml
Sperm Count

- Decreased:
  - vasectomy (should be 0 after 3-6 months)
  - varicocele
  - primary testicular failure (Klinefelter's)
  - secondary testicular failure
  - congenital vas obstruction
  - retrograde ejaculation
  - endocrine causes (prolactinemia, low testosterone)