Unit 5
Seminal Fluids

Seminal Fluids - objectives

1. Discuss the major components of seminal fluid with regard to source, function, normal and abnormal appearance.
2. List three (3) reasons for semen analysis.
3. Outline instructions to give to a patient for the correct method for collecting a semen specimen for laboratory analysis.
4. List two (2) methods for identifying a questionable fluid as semen.
5. State the significance of finding increased acid phosphatase in a suspicious fluid.
6. Calculate a sperm count when provided with the number of sperm counted, the dilution factor and the area of the counting chamber used.
7. List the normal values for: semen volume, viscosity, pH, sperm count, motility and morphology.

Seminal Fluids

- Composition of Semen
  - Spermatozoa
  - Fluids to provide nutritional support and media

<table>
<thead>
<tr>
<th></th>
<th>% of total</th>
<th>Description / Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatozoa</td>
<td>2-5%</td>
<td>Formed in testes, stored in epididymis and vasa differentia</td>
</tr>
<tr>
<td>Seminal Fluid</td>
<td>60-75%</td>
<td>Alkaline fluid; primarily responsible for nutritional support through: amino acids, enzymes, fructose. Also to suppress possible immune response by female</td>
</tr>
<tr>
<td>Prostate Fluid</td>
<td>25-30%</td>
<td>Acid phosphatase, citric acid, proteolytic enzymes and zinc</td>
</tr>
<tr>
<td>Bulbourethral glands</td>
<td>1 - 5%</td>
<td>Galactose, mucous</td>
</tr>
</tbody>
</table>
Seminal Fluids

• Anatomy, composition and formation.
  • Testes - source of sperm (2-5%)
  • Seminal vesicles - provides fructose & nutrients and is primary provider of fluid (@ 60-75%)
  • Prostate gland - Provides enzyme, acid phosphatase, citric acid, zinc, and proteolytic enzymes (for coagulation and liquefication). 2nd source of fluid(25-30%)
  • Bulbourethral glands - @ 5%. Thick alkaline mucous-like fluid that neutralizes acids.

• Spermatozoa - produced in the testes, mature in the epididymis.

Seminal Fluids

• Reasons for Testing
  • Infertility issues - more often a problem with the woman, but easy to rule-out the male.
    • With assisted reproductive technology, greater emphasis placed on sperm quality and quantity.

  • Post-vasectomy - frequent reason for testing
    Test at one month intervals until 2 consecutive months are negative for sperm.
Seminal Fluids

- **Reasons for Testing**
  - Forensic analysis of fluid as being semen
    - as in alleged rape.
    - Vaginal swab, washings, or scrapings microscopically evaluated for sperm
  - Chemical test for enzyme: acid phosphatase
    - Contributed by prostate gland
    - Present even in the absence of sperm cells
  - Sperm donors - artificial insemination programs

- **Specimen Collection**
  - Sterile container
  - Direct deposit preferred
    - no lubricants, spermicides, condoms, etc.
  - Complete specimen
    - Majority of sperm are in first part of ejaculate
  - 3 day sexual abstinence required
    - But not more than 5 days.
  - **Best if collected at laboratory site.**
    - If other, specimen must be kept warm and delivered to lab within 1 hour
  - Time of collection important.
    - Must be recorded!

- **Physical characteristics**
  - Liquefaction - fresh specimen will clot, then liquefy within 30 - 60 minutes
  - Persistence of clot is abnormal
  - **All further evaluation must wait until liquefaction is complete.**
### Seminal Fluids

#### Semen: Appearance

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opaque</td>
<td>Normal</td>
</tr>
<tr>
<td>Gray, white, light yellow</td>
<td>Shades of yellow Correlate with flavin concentration Could also indicate contamination with urine.</td>
</tr>
<tr>
<td>Deep yellow</td>
<td>Associated with certain drugs</td>
</tr>
<tr>
<td>Brown or red</td>
<td>May contain blood</td>
</tr>
<tr>
<td>Highly turbid</td>
<td>Usually contains leukocytes indicating infection or inflammation</td>
</tr>
</tbody>
</table>

#### Semen: Appearance, cont.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>2.0 – 5.0 mL</td>
<td>Measured in serological pipet Recorded to 1 decimal place</td>
</tr>
<tr>
<td>pH</td>
<td>7.2 – 8.0</td>
<td>Measured with pH paper Alkaline to off-set acid vaginal environment Acid may indicate increased prostatic fluids pH &gt; 8 may indicate infection</td>
</tr>
</tbody>
</table>

#### Semen: Appearance, cont.

<table>
<thead>
<tr>
<th>Viscosity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pours in droplets (as shown in picture) Rating: 0 = water-like 4 = gel-like</td>
<td></td>
</tr>
</tbody>
</table>
Semen: Microscopic Analysis

- Motility
- Concentration / cell count
- Morphology
- Agglutination
- Viability
- Penetration of cervical mucous

Microscopic examination

- Generally performed 30 - 60 min after collection
- Must be after liquidification has occurred
  - Motility
    - Motility is a very necessary quality of sperm. Must propel through uterus & fallopian tubes which is quite a long distance.
    - Must be evaluated within the 1st hour following collection
    - Will decrease over time

Semen: Sperm Motility

- Analysis to begin within 1 hour of specimen collection
- Evaluation times may vary between labs, but usually at set intervals
- Consistency in technique and procedure important
- Using hemocytometer & coverslip, examine a drop of undiluted specimen using high dry objective.
- Brightfield microscopy with light level reduced
- Some labs use phase microscopy
- Alternate method: High-resolution video photography / CASA (computer assisted semen analysis)
Semen: Sperm Motility

- **Manual Subjective evaluation**
  - Observe immediately following liquidification, and within 1st hour.
  - Place well mixed undiluted drop on **pre-warmed** hemacytometer slide
  - Observe under high-dry objective; with reduced light.
  - Rated from '0' to 4.0'
    - 4.0 – rapid and straight line movement
    - 3.0 – slower, and some lateral movement
    - 2.0 – slow forward progression, noticeable lateral
    - 1.0 – no forward progression
    - 0 – no movement
  - Other types of rating scales may be used
  - Normal (authors vary greatly) but > 50-60% show 2.0 or greater at 1 hour.

Semen: Microscopic Analysis

- **Morphology**
  - May be performed in cytology, pathology, or hematology
  - Oval/egg shaped head (3x5um)
    - While oval from the front, appears flattened when viewed from the side appears flattened
    - @ ½ covered with an enzyme laden acrosomal cap, which contains
  - Middle piece
    - provides energy
  - Tail piece of @ 45 – 55 um long

Semen: Microscopic Analysis

- **Morphology**
  - At least 200 cells evaluated on smear (Wright's, Giemsa or Papanicolaou) stained.
  - **Usually evaluated by pathologist, or cytologist**
  - Looking for double heads, pin heads, giant heads, or amorphous heads, double, coiled, or missing tails, etc.
  - Many sources of good pictures available
Semen: Microscopic Analysis

- Morphology
  - Normal = < 30% abnormal forms
    (NV varies considerably based on strictness of criteria.
  - WBC, RBC, bacteria presence should be noted & may indicate infection
  - Round cells (neutrophils and immature sperm) should be noted as well.

Semen: Abnormal forms

- 2 headed sperm
  - Sternheimer - Malbin stain
  - X 320
- Flat-headed sperm

Semen: Microscopic Analysis

- Sperm count
  - NV= 20 - 160 million/mL
  - Make 1 to 20 dilution with sodium bicarbonate and formalin, count 5 small squares (within the center large square) of the Neubauer hemacytometer.
Semen: Microscopic Analysis

• Sperm cell count
  • standard method to begin calculation of # cells (mature sperm) per microliter:

\[
\text{ave. # cells counted} \times \text{dilution} \times \text{# squares counted} \times \text{volume of each square}
\]

Semen: Microscopic Analysis

• Microscopic examination
  • Example: 52 cells (mature sperm) x 20
    • 5 (squares) x 0.004

This provides results as ___ cells / uL; normal values are reported as ___ cells / mL

• Must multiply X 1000 to convert uL to mL
  • = 52.0 x 10^6 / mL

Semen: Microscopic Analysis

• Metric
  • Internationalized system using decimals
  • Common system of measuring units
    • Length (M), volume (L), mass (G), time (s), temperature (°C)
    • Prefixes allow for mL, uL, etc.
  • International System of Units (SI)
    • Modified / modern form of metric system
    • Has 7 base units (but, unlike the original metric system does not include volume)
    • Other units, such as volume are 'derived'
    • Basic unit for volume is m^3
    • mL = cubic centimeter (cm^3), uL = cubic millimeter (mm^3)
Semen: Sperm Agglutination

- Observed while performing motility evaluation.
- Few clumps are normal.
- Distinctly head-to-head or tail-to-tail clumping may indicate the presence of antisperm antibodies.
  - IgG
  - IgA

Semen: Sperm Viability

- Eosin – Nigrosin stain
  - supravital stain
  - Add to drop of fresh sample
  - Smear is made and allowed to dry
  - Evaluated on oil immersion (1000x)
  - Viable / live sperm do not take up the stain and remain colorless or blue-white
  - Non-viable / dead sperm stain orange-red
- Reported as % viable
- Normal >75%

Semen: Analysis

- Other tests
  - Sperm penetration
    - Evaluates ability of sperm to make progressive movement through the cervical mucous.
  - Microbial testing
    - Increased WBC (>1 million/ mL) suggestive of infection
    - Aerobic and anaerobic cultures
Semen: Chemical Analysis

- **pH**
  - Measure within 1 hour of collection
  - Normal 7.2-8.0

- **Acid Phosphatase**
  - Used to evaluate the secretory function of the prostate
  - Also used in forensic analysis – as prostatic fluid acid phosphatase is higher than other fluids (>200 units)

- **Fructose**
  - Provides energy / nutrition to sperm
  - Indication of viability
  - Presence of fructose – screen using resorcinal test

- **Hormones**
  - Testosterone, LH, FSH

Post- Vasectomy Analysis

- Post-vasectomy semen analysis
  - Specimens tested at monthly intervals starting 2 months post-vas.
  - 2 consecutive months of negative microscopic for sperm
  - Wet prep with phase microscopy
  - Examination of centrifuged specimen as well

Semen: Forensic Analysis

- Examination of fluid as to being semen (forensic)
  - Acid phosphatase – highly sensitive, as no other body fluid contains as high level (2500 units compared to @ 5 units)
  - ABO, HLA typing
  - DNA analysis
  - UV light scan, semen fluoresces green/white
Semen: Analysis QC

- Quality control
  - Previously little or no QC materials available
  - Commercial products now becoming available
  - Proficiency testing now available
    - CAP
    - American Association of Bioanalysts (AAB)

Reference Listing

- Please credit those whose work and pictures I have used throughout these presentations.
- Lillian Mundt & Kristy Shanahan, Greff's Textbook of Urinalysis and Body Fluids, 2nd Ed.
- Susan Strassinger & Marjorie Di Lorenzo, Urinalysis and Body Fluids, 5th Ed.
- Wikipedia, the free encyclopedia
  - www.wikipedia.org