Urinalysis and Body Fluids

Unit 5
Seminal Fluid

Seminal Fluids

- Anatomy, composition and formation.
  - Testes - source of sperm
  - Seminal vesicles - provides fructose & nutrients and is major provider of fluid.

- Anatomy cont.
  - Prostate gland - Provides enzyme, acid phosphatase, citric acid, zinc, and proteolytic enzymes (for coagulation and liquefication). 2nd source of fluid.
  - Bulbourethral glands - ~ 5%. Thick alkaline mucous-like fluid that neutralizes acids.
Seminal Fluids

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

• 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.

• 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 (discussed later)

• Sperm donors - artificial insemination programs
Seminal Fluids

• Specimen Collection
  • Sterile container
  • - direct deposit preferred
  • - no lubricants, spermacides, condoms, etc.
• 3 day sexual abstinence required (but not more than 5 days)
  • Ideal collection site is at laboratory
  • If other, specimen must be kept warm and delivered to lab within 1 hour
  • Time of collection important. (& must be recorded)

Seminal Fluids

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

Seminal Fluids

• Appearance
  • Grey-white, translucent, characteristic musty odor
    • Increased white turbidity indicates increased WBC and likely infection (leukocyte esterase test - part of UA dipstick, may be useful)
    • Red coloration indicates RBCs
    • Yellow coloration can indicate urine contamination, medications, prolonged abstinence.
Seminal Fluids

- Appearance cont.
  - Volume NV = 2-5 mL
  - Viscosity - from a pipette tip, specimen should pour in droplets, and not be too stringy
    - Rating 0 = water-like through 4+ gel-like
    - Increased viscosity &/ incomplete liquefaction will reduce sperm motility
  - pH NV = @ 7.2 - 8.0 (check your lecture guide)
    - Alkaline to offset the vaginal environment
  - Sperm count NV = 20-160 million / mL

Seminal Fluids

- Microscopic examination - done 30 - 60 min after collection 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.
    - Using a hemocytometer, examine a drop of undiluted specimen using high dry objective

Seminal Fluids

- Microscopic examination
  - Motility
    - Subjective evaluation
      - Rated from "0" to 4.0
      - See table 11-2
      - 4.0 - rapid and straight line movement
      - 3.0 - slower, and some lateral movement
      - 2.0 - slow forward progression, noticeable lateral movement
      - 1.0 - no forward progression
      - 0 - no movement
Seminal Fluids

- Microscopic examination
  - Motility
    - Evaluation times may vary between labs, but usually at set intervals
    - Consistency in technique and procedure important
    - NV = 50% showing 2.0 or greater motility at 1 hour after collection
    - New technology - CASA (computer assisted semen analysis) provides objective evaluation, but only found in specialized labs.

- Microscopic examination
  - Morphology
    - Oval/egg shaped head (3x5um) which is half covered with an enzyme laden acrosomal cap
    - Middle piece which provides energy
    - Tail piece of @ 45 um long

- Microscopic examination
  - 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. (see table in text)
Seminal Fluids

- Microscopic examination
  - 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.

- Abnormal, 2 headed sperm
  - Sternheimer - Malbin stain
  - X 320

- 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.
Seminal Fluids

- Microscopic examination
  - standard method to begin calculation of # cells (mature sperm) per microliter:
    \[
    \frac{\text{ave. # cells counted} \times \text{dilution}}{\text{# squares counted} \times \text{volume of each square}}
    \]

- Example: 52 cells (mature sperm) \( \times 20 \)
  - \( 5 \) (squares) \( \times 0.004 \)

  This provides results as ____ cells / uL.
  Normal values are reported as ____ cells / mL

  Must multiply \( \times 1000 \) to convert uL to mL
  \( = 52.0 \times 10^6 / \text{mL} \) (check my math)

Seminal Fluids

- Other tests (infertility)
  - Sperm viability - smear made from specimen mixed with eosin-nigrosin stain and evaluated under microscope.
  - Living cells appear blue-white
  - Dead cells appear red against blue background
  - Authors vary in normals, but at least 50-75% should be live cells
Seminal Fluids

- Other tests
  - Seminal fluid fructose level
  - Indication of viability
  - Presence of fructose screened using resorcinol test
  - Sperm antibodies (either male or female)
  - See textbook for specifics of testing
- Microbial testing
  - Increased WBC (>1 million/mL) suggestive of infection
  - Aerobic and anaerobic cultures
  - Additional chemical testing described pg. 175

Seminal Fluids

- 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

Seminal Fluids

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

- 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