

Urinalysis and Body Fluids CS

Unit 5 2 Pregnancy & Amniotic Fluid Testing

Pregnancy Testing & Amniotic Fluid - objectives

1. Describe HCG, explain its role in pregnancy testing, and identify causes for false negative and false positive results.
2. Analyze the formation, composition, and physiology of amniotic fluid.
3. State three (3) reasons for amniotic fluid analysis.
4. Describe tests performed on amniotic fluid to determine risk of Hemolytic Disease of the Fetus and Newborn (HDFN) and fetal maturity.
5. Define amniocentesis and list special precautions needed for this procedure.
6. Describe the handling and processing procedures for testing amniotic fluid.
7. Explain the principle of spectrophotometric analysis of amniotic fluid for bilirubin and the interpretation of results as to level of risk to the fetus.
8. Evaluate the L/S ratio including its significance and normal value in a mature fetus.
9. Identify the significance of phosphatidylglycerol and the "foam" or "shake" test.
10. Explain the significance of alpha fetal protein and cytogenetic analysis of amniotic fluid.

Urine Pregnancy Testing

- Pregnancy tests detect the hormone produced in pregnancy, beta-human chorionic gonadotropin hormone (β -hCG).
 - A natural hormone produced in very small quantities in all persons, male / female.
- Following fertilization of an ovum, the special cells in the chorionic layer of the developing placenta produce increased amounts.
 - Levels essentially double daily, until a peak level is normally reached near the end of the first trimester.
 - The hormone begins showing up in the urine @ 8-10 days after fertilization (2-3 days after implantation of the embryo)

Urine Pregnancy Testing

- Pregnancy tests detect the increased amount of, beta-human chorionic gonadotropin hormone (β -hCG).
- Pregnancy testing can be done on blood or urine.
 - Blood levels detected earlier and are more constant
 - Urine levels vary depending upon the state of hydration (first morning specimen or one that has SpGr \geq 1.015)

Urine Pregnancy Testing (cont.)

- Enzyme immunoassays (EIAs) are most popular methodology.
 - Can show a positive in as little as 10 days after conception.
 - Example of how results are reported: hCG negative or hCG positive
- False results can occur
 - False negatives - generally due to testing too early
 - False positives
 - Misinterpretation of results , not following directions
 - Contamination with large amounts of blood, protein, bacterial contamination
 - True positive, but patient NOT pregnant
 - Trophoblastic tumors, choriocarcinoma, germ cell tumors, etc.

Amniotic Fluid

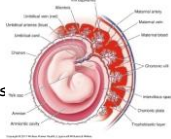
Physiology, Composition and Formation

- Contained within the amnion (The membranous sac surrounding the fetus).
- Function
 - Provides protective cushion
 - Allows exchange of water, nutrients, biochemical products
- Formed by
 - Maternal circulation/plasma (early)
 - Transfer of water across placental membrane
 - Metabolism of fetal cells
 - Fetal urine
 - (after @ 36 weeks)

Amniotic Fluid

Physiology, Composition and Formation

- Volume
 - 700-1200 mL @ 34 weeks.
- Composition
 - Similar to maternal plasma with sloughed fetal cells.
 - Fetal urine increases creatinine, urea & uric acid
 - Rise in creatinine levels after 36th week can be used to evaluate fetal age
 - < 36 weeks = 1.5 - 2.0 mg/dL
 - > 36 weeks = > 2.0 mg/dL
 - Fetal lung secretions
 - Lecithin & sphingomyelin surfactants

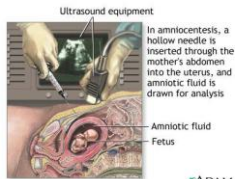


Amniotic Fluid

- Indications for analysis
 - Abnormal screening blood tests: maternal alpha fetal protein, human chorionic gonadotropin, unconjugated estriol
 - Metabolic disorders, *such as Tay Sachs*
 - Neural tube defects - *such as spinal bifida or an encephalic*
 - Abnormal chromosome analysis and history of genetic disorders - *such as Down's syndrome*
- Abnormal ultrasound
- In later pregnancy for possible early delivery
 - Fetal lung maturity, hemolytic disease of the newborn (HDN), infection, confirmation of gestational age, fetal maturity, etc.

Amniotic Fluid

- Specimen collection
 - Amniocentesis (using ultrasound)
 - 16-42 weeks gestation
 - @ 20-30 mL through fine needle collected in sterile syringes
 - **Immediately transfer into sterile tubes (brown colored for protection from light)**
 - Puncture heals and liquid replenished within 48 hrs.



Amniotic Fluid

- **General Handling and processing**
- **Special precautions**
 - Cytogenic study specimens at RT or 37 degrees
 - Fetal lung maturity testing specimens must be kept cold until tested
 - Specimens for bilirubin testing must be *protected from light exposure and process immediately*
 - Other chemistry tests require separation of cells, etc. from the fluid to preserve constituents.

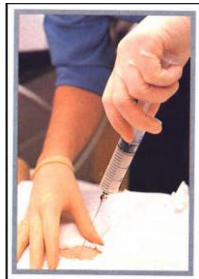
Amniotic Fluid

- **Color & Appearance**
 - Normally colorless - pale yellow
 - Some turbidity is normal - cellular debris, especially late in fetal development period.



Amniotic Fluid

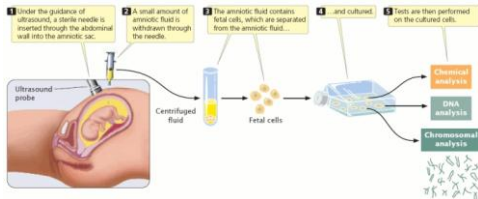
- **Color & Appearance cont.**
 - Blood streaked - traumatic tap, abdominal hemorrhage, intra-amniotic hemorrhage
 - Fetal blood vs maternal blood: use Kleihauer-Betke
 - Yellow - bilirubin
 - Dark green- meconium
 - Dark red-brown -
 - probable fetal death has occurred.



Amniotic Fluid testing

Cytogenetic analysis

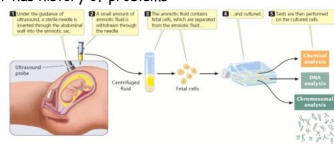
- Determination of chromosomal abnormalities and certain metabolic defects
 - Picture by Clare O'Connor, PhD, Biology Dept., Boston College.
 - Prenatal Screens Detects Fetal Abnormalities., [Nature Education](#).



Amniotic Fluid testing

Cytogenetic analysis

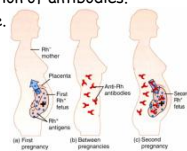
- Determination of chromosomal abnormalities and certain metabolic defects
- Cells cultured
 - Chromosomess evaluated for appropriate number and completeness
 - Some cells lysed and contents analyzed for enzymes to evaluate for metabolic defects, such as Tay Sachs.
- Not done on all patients
 - Patient is >35yrs. or has history of problems
 - Increased AFP
 - Known carriers.



Amniotic Fluid testing in HDN

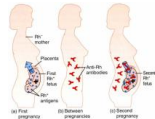
Hemolytic Disease of the Newborn (HDN)

- Also called Hemolytic Disease of the Fetus and Newborn (HDFN) or erythroblastosis fetalis.
- "classical" case: Rh-negative mothers with Rh+ infants
- Other red blood group discrepancies between Mom and Baby can also produce HDN
- Fetal cells with antigen foreign to the Mom enter her circulation and stimulate the production of antibodies.
- Danger increases with each exposure.



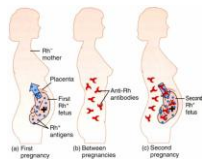
Amniotic Fluid testing for Fetal Distress

- Hemolytic Disease of the Newborn (HDN)
 - Maternal antibodies cross the placenta and destroy fetal cells with the corresponding antigen
 - Bilirubin from RBC destruction appears in the amniotic fluid
 - Some level occurs naturally from normal RBC catabolism
 - The amount of unconjugated bilirubin present correlates with the amount of RBC destruction



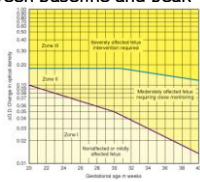
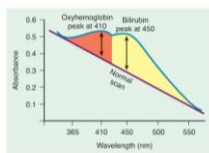
Amniotic Fluid testing for Fetal Distress

- Purpose of bilirubin testing on amniotic fluid:
 - *Measurement of bilirubin is an indication of degree of hemolysis occurring in utero, therefore an indication of danger of anemia in the fetus.*



Amniotic Fluid testing for Fetal Distress

- Bilirubin by spectrophotometric analysis
 - Scan fluid at increasing wavelengths
 - Plot readings against a baseline
 - Measure difference between baseline and peak bilirubin at 450 nm



Plot difference on Liley graph, against gestational age

Amniotic Fluid Testing for Neural Tube Defects

- Neural Tube Defects
 - Anencephaly
 - Spinal bifida
- Tests (these are usually tested together)
 - Alpha fetal protein (AFP)
 - Peaks at 16 weeks gestation
 - Acetylcholinesterase
 - **Test affected by presence of blood or hemolysis

Amniotic Fluid Testing for Fetal Lung Maturity

- To determine whether fetus is capable of surviving an early delivery.
 - Hyaline membrane disease
 - Also called neonatal respiratory distress syndrome
 - Major complication of early delivery & **Most common cause for death of premature newborn.**
 - Immature lungs lack of lung surfactants, which allow lung alveoli to be able to open during when breathing.
 - Surfactants decrease surface tension, permitting alveoli inflation.
 - Fetal lung surfactants include three phospholipids:
 - lecithin (also known as phosphatidylcholine). **major lung surfactant.
 - sphingomyelin,
 - phosphatidyl glycerol.
 - Many tests developed to assess for fetal lung maturity.

Amniotic Fluid Testing for Fetal Lung Maturity

- Shake test & Foam stability
 - Both tests utilize dilutions of amniotic fluid in 95% ethanol and look for formation of a persistent ring of foam / bubbles, an indication of total surfactant concentration.
 - **Shake test** - crude, fast, cheap and can be performed at bedside. Physician can make an immediate decision regarding safety of early delivery of infant.
 - 1:2 dilution (amniotic fluid : 95% ethyl alcohol), shake 15 seconds, If a complete ring of foam persists 15 minutes = positive test.
 - **Foam stability index** - places fixed amount of amniotic fluid in series of tubes with increasing amounts of 95% ethanol (from 0.43 to 0.55)
 - Dilutions are shaken vigorously and the higher concentration of 95% alcohol that is able to suppress a foam ring is known as the foam stability index
 - Foam stability index ≥ 0.47 indicates fetal lung maturity

Amniotic Fluid Testing for Fetal Lung Maturity

- Lamellar bodies
 - "packets" of surfactant lipids produced by pneumocytes
 - Size 1-5 um (slightly smaller than platelets)
 - Can run on automated cell counters (platelet mode)
 - Lamellar body count > 30,000 / uL is highly predictive of pulmonary maturity
 - Count < 10,000 suggest risk for RDS - respiratory distress syndrome
 - Test not affected by hemolyzed blood or meconium

Amniotic Fluid Testing for Fetal Lung Maturity

- Lecithin / Sphingomyelin ratio
 - Lecithin is the major lung surfactant
 - The role of sphingomyelin is not established
 - Levels equal until 33 weeks of gestation
 - @ 1/1 ratio
 - After 34 the week of gestation, lecithin production greatly increases as compared to the sphingomyelin
 - L/S ratio 2.0 or greater indicates lung maturity
 - 2X as much lecithin / sphingomyelin

Amniotic Fluid Testing for Fetal Lung Maturity

- Phosphatidyl glycerol
 - Not detected until 35 week of gestation
 - Delayed in cases of maternal diabetes
- Amniostat FLM - PG
 - Immunological test for phosphatidyl glycerol
 - Uses antibodies for detection, thereby not affected by the presence of hemolysis or blood

Amniotic Fluid Testing for Fetal Lung Maturity

- Microviscosity
 - Fluorescent dye binds with surfactants and albumin.
 - Test run on Abbot TDx and results correlate well with L / S ratio

Amniotic Fluid Testing

- Creatinine
 - Fetal age determination (*at 36 weeks, fetal kidneys excrete >2.0 mg/dL creatinine*)
 - *This test has been replaced by ultrasound measurements.*
- Creatinine still used as:
 - Measurement as means of determining a fluid to be amniotic or urine.
 - Creatinine level up to 3.5 mg/dL & urea level @ 30 mg/dL can be found in amniotic fluid
 - Urine levels of creatinine @10 mg/dL and @ 300 mg/dL for urea.

Reference Listing

- > Please credit those whose work and pictures I have used throughout these presentations.
- > Lillian Mundt & Kristy Shanahan, Graff'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
- > National Library of Medicine's Medline Plus
 - > <http://www.nlm.nih.gov/medlineplus/ency/imagepages/1062.htm>
- > Sitable by Nature Education, a collaborative learning space for science.
 - > <http://www.nature.com/scitable/topicpage/prenatal-screen-detects-fetal-abnormalities-306>
- > The Amniostat-FLM -PG, Irvine Scientific
 - > http://www.irvinesci.com/techinfo/docs/AmnioStat-FLM-PG_Rev0.pdf
