Complications of Childbirth

David G. Grenache, PhD
University of Utah & ARUP Laboratories
Salt Lake City, UT
Disclosures

• Abbott Point of Care, Inc.
  – Grant/Research Support
Objectives

• Describe the tests used to identify premature rupture of membranes

• Explain the strengths and limitations of fetal fibronectin tests for predicting preterm birth

• Compare and contrast the two commercially available test for assessing fetal lung maturity

• Describe testing strategies used to assess HIV maternal and fetal HIV status
Premature Rupture of Membranes
**PROM vs. PPROM**

**Preivable Premature Rupture of Membranes**
- 14 to 24 weeks of gestation
- Occurs in <1% of all pregnancies
- Poor prognosis
- Survival more likely after 22 weeks

**Preterm Premature Rupture of Membranes (PPROM)**
- 24 to 37 weeks of gestation
- Occurs in 3% of all pregnancies
- Responsible for 30-40% of preterm births
- 50% deliver within 7 days regardless of management

**Premature Rupture of Membranes (PROM)**
- Ruptured membranes before the onset of labor at or beyond 37 weeks of gestation
- Occurs in ~10% of all pregnancies
- Usually followed by spontaneous labor and delivery (95% w/in 28 h)

ACOG. Obstet Gynecol 2013;122:918-930
Etiology of ROM

- Apoptosis
- Activation of MMPs
- Mechanical Forces

Ruptured Membranes

Strauss JF. Reprod Sci 2013;20:140-153
Clinical Features of PPROM

• Risk factors*
  – Previous PPROM
  – Genital tract infection
  – Cigarette smoking
  – Vaginal bleeding in any trimester
  – Low socioeconomic class
  – Short cervical length
  – Low BMI

*similar to those associated with preterm birth

• Patient presentation
  – Sudden "gush" of clear or pale yellow fluid from the vagina
  – May be intermittent or constant leaking of small amounts of fluid

ACOG. Obstet Gynecol 2013;122:918-930

https://www.youtube.com/watch?v=eP-MiUKQVR8
Diagnosis of PROM

• ~90% of cases identified by clinical observation
  – Visualization of amniotic fluid in vagina

• Additional testing when diagnosis is uncertain
  – Gold standard: indigo carmine dye injected into amniotic cavity
    • pH indicator (blue at <11; yellow at >13)
    • Vaginal tampon examined for blue staining
  – Laboratory testing
pH/Nitrazine Tests

- Amniotic fluid pH (7.0 to 7.7) is higher than the normally acidic vagina (3.8 to 4.2)
- False-positives due to the presence of alkaline fluids (e.g. blood, semen, soap)
- False-negatives due to prolonged rupture and intermittent/small leaks

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Population</th>
<th>n</th>
<th>pH Cutoff</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(53)</td>
<td>1995</td>
<td>Membrane status known</td>
<td>51</td>
<td>≥7.0</td>
<td>100</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Membrane status unknown</td>
<td>40</td>
<td></td>
<td>72</td>
<td>64</td>
</tr>
<tr>
<td>(54)</td>
<td>1990</td>
<td>Membrane status known</td>
<td>45</td>
<td>&gt;6.0</td>
<td>91</td>
<td>73</td>
</tr>
<tr>
<td>(56)</td>
<td>1995</td>
<td>Membrane status known</td>
<td>103</td>
<td>&gt;6.5</td>
<td>92</td>
<td>53</td>
</tr>
<tr>
<td>(58)</td>
<td>1977</td>
<td>Membrane status known</td>
<td>39</td>
<td>Not given</td>
<td>100</td>
<td>92</td>
</tr>
<tr>
<td>(59)</td>
<td>1987</td>
<td>Membrane status known</td>
<td>79</td>
<td>≥7.0</td>
<td>77</td>
<td>81</td>
</tr>
<tr>
<td>(60)</td>
<td>1995</td>
<td>Membrane status known</td>
<td>30</td>
<td>Not given</td>
<td>100</td>
<td>41</td>
</tr>
</tbody>
</table>

NACB. Practice Guideline: Evidence Based Practice for Point-of-Care Testing 2006
Fern Test

• Fluid from the posterior vaginal fornix is swabbed onto a glass slide, air dried, and examined microscopically for ferning pattern

• ~50% sensitive; 70% specific (de Hann HH, et al. *Am J Perinatol* 1994;11:46-50)

• NACB: “The fern test is neither sensitive nor specific enough for diagnostic determination of PROM.”

---

UpToDate, 2015
Placental α Microglobulin-1 (PAMG-1)

• Glycoprotein secreted by placenta
  - AF: 2,000-25,000 ng/mL
  - Maternal blood: 5-25 ng/mL
  - Cervicovaginal fluid (intact membranes): 0.05-2 ng/mL

• Commercially available immunochromatographic test (AmniSure® ROM)

http://www.aghealth.co.uk
Insulin-like Growth Factor Binding Protein 1 (IGFBP-1)

• Glycoprotein secreted by several tissues

• Predominant binding protein of IGF fetal and maternal blood and AF
  – AF: 10,000-400,000 ng/mL
  – Maternal blood: 60-600 ng/mL

• Commercially available immunochromatographic test (Actim® PROM)
  – ~77% sensitive; 80% specific in equivocal cases
Management of PPROM

• Controversial and based on several factors
  – Gestational age
  – Availability of neonatal intensive care
  – Presence/absence of infection, labor, placental abruption
  – Cervical status

• Immediate delivery if medical required otherwise expectant management
Clinical history of PPROM

Admit to labor and delivery unit

Confirm diagnosis (eg, pooling, ultrasound, Nitrazine and fern tests, Amnisure)

Overt maternal infection or nonreassuring fetal status

Deliver

Stable maternal and fetal status

Hospitalize until delivery

Administer antenatal corticosteroids, prophylactic antibiotics, monitor for infection and fetal well-being

At 34 weeks, confirmed gestational age

Deliver

At 34 weeks, unconfirmed gestational age

Check fetal lung maturity

Mature lung profile

Deliver

Immature lung profile

Expectant management with delivery at 36 weeks
Preterm Birth
Preterm Birth

- Delivery before 37 weeks of gestation
- 11.7% of US births in 2011 were preterm
- Leading cause of neonatal death (death <29 days)
  - 27% of neonatal deaths worldwide (>1M annual deaths)
- Majority of surviving preterm infants suffer serious morbidities
  - Cerebral palsy, chronic lung disease, gastrointestinal problems, mental retardation, vision or hearing loss
- Incidence rising in nearly all countries
Types of Preterm Birth

- Spontaneous labor: 50%
- PPROM: 30%
- Medically necessary: 20%

Preterm Birth Risk Factors
(partial list)

• Smoking
• Alcohol consumption
• Poor nutrition/low BMI
• Advanced maternal age
• Low socioeconomic status
• Anxiety/depression
• Multiple gestations
• Polyhydramnios

• Sexually transmitted infections
• Systemic infection
• Previous preterm birth
• Race (highest in Blacks)
• Inadequate prenatal care
• Fetal anomaly/growth restriction
• Environmental factors

Etiology of Preterm Birth

- Myometrial contraction
- Membrane activation
- Cervical ripening

Factors:
- Autoimmune & allergy
- PPROM
- Infection/inflammation
- Decidual hemorrhage
- Uterine distension
- Environmental factors
- Behavioral/socioeconomic factors

Adapted from Gracie S, et al. BMC Pregnancy and Childbirth 2011;11:71
Symptoms of preterm labor are neither sensitive nor specific

- Menstrual-like cramping
- Change in vaginal discharge
- Backache
- Abdominal discomfort
- Pelvic pressure
- Cramping

True or false labor?  
Who is likely to deliver preterm?

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical dilation &gt;1 cm</td>
<td>29%</td>
<td>82%</td>
<td>11%</td>
<td>94%</td>
</tr>
<tr>
<td>Contraction frequency &gt;7/h</td>
<td>42%</td>
<td>67%</td>
<td>9%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Fetal Fibronectin (fFN)

- Member of the fibronectin family (large ECM glycoproteins)
  - Unique epitope due to differential glycosylation
  - Recognized by MAb FDC-6

- Trophoblast “glue”
  - Promotes cell adhesion at uterine-placental and decidual-chorionic interfaces

- Released into cervicovaginal fluids when ECM of decidual-chorionic interface is disrupted

http://www.ffntest.com
fFN and Gestational Age

Lockwood CJ, et al. NEJM 1991;325:669-674
Indications for fFN Use

• Symptomatic Women
  – 24⁰-34⁶ weeks of gestation
  – Assess risk of delivery in <7 or <14 days from testing

• Asymptomatic Women
  – 22⁰-30⁶ weeks gestation
  – Aid in assessing risk of delivery <35 weeks of gestation

• Contraindicated
  – Advanced cervical dilation (>3 cm)
  – Rupture of amniotic membranes
  – Cervical cerclage
  – Moderate or gross vaginal bleeding
  – Sexual intercourse in preceding 24 hrs
fFN Test

- Cervicovaginal fluid

- Immunochromatographic method
  - Mouse monoclonal anti-fFN (signal)
  - Goat polyclonal anti-FN (capture)
  - Signal interpreted by the TLiIQ® Analyzer
    - Positive (≥50 ng/mL)
    - Negative (<50 ng/mL)
    - Invalid

http://www.hologic.com
fFN in Symptomatic Women

- A “good test” has a LR+ >10 or LR- of <0.1

- Assuming a PTB prevalence of 12%
  - 37% PPV
  - 96% NPV

- Is this a useful test?
  - High NPV is potentially valuable to identify symptomatic women unlikely to deliver preterm

fFN in Asymptomatic Women

• Most experts recommend against fFN testing in asymptomatic women
  – fFN performance is poor in this population
  – Few potentially effective interventions are available

• ACOG: “Tests, such as fetal fibronectin...are not recommended as screening strategies”

ACOG. Obstet Gynecol 2012;120:964-973
Fetal Lung Maturity
Fetal Lung Development

http://www.embryology.ch/anglais/rrespiratory/phasen01.html
Surface Tension & Surfactant

- Internal surface of the alveolus is covered with a thin coating of fluid

- Water in this fluid has a high surface tension which promotes collapse the alveolus

- Pulmonary surfactants decrease surface tension of water
  - Increases lung compliance
  - Prevents collapse of alveoli during expiration
Surfactant Metabolism

Pulmonary Surfactants

- Phosphatidylcholine (76%)
  - AKA lecithin
- Phosphatidylglycerol (13%)
- Phosphatidylinositol (4%)
- Phosphatidylethanolamine (3%)
- Sphingomyelin (2%)
- Other phospholipids (2%)

Composition:
- Phospholipid: 85%
- Neutral lipid: 10%
- Protein: 5%
Respiratory Distress Syndrome (RDS)

- Was known as hyaline membrane disease
- Caused by a deficiency in pulmonary surfactant
- Most common cause of respiratory failure in neonates
- Incidence is indirectly proportional to gestational age at delivery

Pathophysiology of RDS

Reduced surfactant

Impaired cellular metabolism

Alveolar collapse

Fibrin + necrotic cells (hyaline membrane)

Alveolar hypoperfusion

Plasma leaks into alveoli

Hypoperfusion

Epithelial damage

Pulmonary vasoconstriction

Hypoxemia, hypercapnia, resp. acidosis

Hypoxemia, hypercapnia, resp. acidosis
Prevention/Treatment of RDS

- Antenatal prevention
  - Prevention of preterm delivery
  - Maternal administration of corticosteroids stimulates fetal synthesis of surfactant

- Surfactant replacement
  - Prophylaxis of preterm infants at risk for RDS
  - Treatment of infants with RDS

- Continuous positive airway pressure (CPAP) or assisted ventilation
  - Keeps alveoli open at expiration
Tests for Fetal Lung Maturity (FLM)

• Performed on amniotic fluid
  – 32-38^{6/7} weeks of gestation

• Used for decision making
  – Allow or delay delivery w/ steroid administration
  – Uncertain gestational age
  – Transfer mother to facility with NICU

• Must have high sensitivity for immaturity & high negative (mature) predictive value

• Performed rapidly (ideally)
FLM Test History


- Foam Stability
- OD 650
- S/A Ratio
- PG by Agglutination
- Lamellar Body Count
- PG by TLC
- L/S Ratio

10 min 120 min
FLM Test History

- 1971: L/S Ratio
- 1972: PG by TLC
- 1979: Foam Stability
- 1980: OD 650
- 1983: S/A Ratio
- 1986: PG by Agglutination
- 1988: Lamellar Body Count

PG by TLC
OD 650
S/A Ratio
Lamellar Body Count
Lecithin/Sphingomyelin (L/S) Ratio

- Thin-layer chromatography
- First test of fetal lung maturity
- Undeserving gold standard
L/S Ratio

Pros
• Good sensitivity for immaturity (~85%)
• Excellent mature predictive value (~95%)
• Commercially available

L/S Ratio Cutoffs (ARUP)

<table>
<thead>
<tr>
<th>Stage</th>
<th>L/S Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>≤1.5</td>
</tr>
<tr>
<td>Transitional</td>
<td>1.6-2.4</td>
</tr>
<tr>
<td>Mature</td>
<td>≥2.5</td>
</tr>
</tbody>
</table>

Cons
• Large sample volume
• Affected by blood and meconium
• Time consuming
• Technically difficult
• Imprecise (CV ~20%)
• Wide grey zone

Lamellar Body Count (LBC)

• LBs similar in size to blood platelets
  – 1 to 5 μm vs. 2 to 4 μm

• Enumerate with automated cell counter

*lamella |laˈmɛlə| Noun
a thin layer, membrane, scale, or platelike tissue or part, esp. in bone tissue
ORIGIN late 17th cent.: from Latin, diminutive of *lamina ‘thin plate.’*
Lamellar Body Count

Pros
• Excellent sensitivity for immaturity (~94%)
• Excellent mature predictive value (~97%)
• Low sample volume
• Technically simple

Cons
• Affected by blood and meconium
• Lab-developed test
• Instrument-specific cutoffs for maturity

Neonatal Outcomes After Demonstrated Fetal Lung Maturity Before 39 Weeks of Gestation

Elizabeth Bates, MD, Dwight J. Rouse, MD, MSPH, Merry Lynn Mann, BS, Victoria Chapman, MPH, Waldemar A. Carlo, MD, and Alan T. N. Tita, MD, PhD

(Obstet Gynecol 2010;116:1288–95)

• Two cohorts
  – 36-38 weeks with mature L/S ratio test result (N=459)
  – 39-40 weeks (N=13,339)

• Delivery before 39 weeks associated with increased risk of adverse outcome

Death, RDS, TTN, bronchopulmonary dysplasia, Persist pulmonary hypertension, supplemental O2, surfactant, other (hyperglycemia, etc), sepsis
Documented fetal lung maturity is insufficient to determine an infant’s readiness for postnatal life.

FLM testing is irrelevant when preterm delivery is medically necessary.

Infants should not be delivered electively at <39 weeks’ gestation even if lung maturity is demonstrated.
Vertical HIV Transmission
HIV in the United States

Vertical HIV Transmission

• Mother-to-infant infection during pregnancy, childbirth, or breastfeeding

• Risk is 25-30% among untreated mothers
  – Reduced to ~2% with proper interventions
    • HIV testing, antiretroviral therapy, C-section delivery prior to onset of labor, no breastfeeding

• HIV screening during pregnancy is essential
  – >1.2M people have HIV infection and 14% (1 in 7) are unaware
  – 30% of pregnant women not tested for HIV during pregnancy
  – 15-20% receive no or minimal prenatal care
HIV Screening During Pregnancy

• Test as early as possible by default unless patient declines
  – Opt-out strategy not permitted in all states (http://nccc.ucsf.edu)

• Repeat test in 3rd trimester for high-risk women
  – IV drug use, STDs during pregnancy, multiple sex partners during pregnancy, live in high HIV prevalence areas, or have HIV-infected partners

• Rapid HIV screening if in labor and undocumented HIV status
  – Immediate antiretroviral prophylaxis provided while result confirmed

ACOG. Obstet Gynecol 2008;112:739-742
3rd & 4th Generation Serological HIV Tests

- **3rd Generation**
  - Detect IgG and IgM anti-HIV-1/HIV-2 antibodies
  - Sensitivity and specificity >99.5%

- **4th Generation**
  - Detect IgG and IgM anti-HIV-1/HIV-2 antibodies
  - Detect HIV p24 antigen
  - Sensitivity >99.8%; specificity >99.5%

Recommended HIV Testing Algorithm

HIV-1/2 antigen/antibody combination immunoassay

(+)  
(-)  

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)  
HIV-2 (-)  
HIV-1 antibodies detected

HIV-1 (-)  
HIV-2 (+)  
HIV-2 antibodies detected

HIV-1 (+)  
HIV-2 (+)  
HIV antibodies detected

HIV-1 (-) or indeterminate  
HIV-2 (-)  

HIV-1 NAT

(+) indicates reactive test result  
(-) indicates nonreactive test result  
NAT: nucleic acid test

HIV-1 NAT (+)  
Acute HIV-1 infection

HIV-1 NAT (-)  
Negative for HIV-1

CDC. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations(2014)  
http://stacks.cdc.gov/view/cdc/23477
Alternatives to Recommended HIV Testing Algorithm

3rd generation HIV-1/2 antibody test

(+)

HIV-1/HIV-2 antibody differentiation immunoassay

HIV-1 (+)  HIV-1 (-)  HIV-1 (+)  HIV-1 (-) or indeterminate
HIV-2 (-)  HIV-2 (+)  HIV-2 (+)  HIV-2 (-)

HIV-1 antibodies detected  HIV-2 antibodies detected  HIV antibodies detected  HIV-1 NAT

(+ indicates reactive test result  (-) indicates nonreactive test result
NAT: nucleic acid test

HIV-1 NAT (+)  HIV-1 NAT (-)
Acute HIV-1 infection  Negative for HIV-1

Negative for HIV-1 and HIV-2 antibodies and p24 Ag

CDC. Laboratory Testing for the Diagnosis of HIV Infection: Updated Recommendations(2014)
http://stacks.cdc.gov/view/cdc/23477
Strategies to Prevent Vertical HIV Transmission

• Maternal 3-drug ART at start of 2nd trimester or earlier regardless of CD4 count or viral load

• Intravenous zidovudine during labor if HIV RNA >400 copies/mL or unknown

• Elective C-section before labor if HIV RNA >1,000 copies/mL or unknown near delivery

• 6-week ART for all HIV-exposed infants

• Avoidance of breastfeeding in all women

ACOG. *Int J Gynaecol Obstet* 2001;73:279-281
http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf
Testing Infants for HIV

• Serological testing not reliable due to maternal transfer of anti-HIV IgG
  – Antibodies can persist for 18 months

• Virologic (nucleic acid) testing to identify infected infants
  – Do not test cord blood (possible maternal contamination)

<table>
<thead>
<tr>
<th>Time</th>
<th>HIV DNA (qual)</th>
<th>HIV RNA (quant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>55%</td>
<td>25-60%</td>
</tr>
<tr>
<td>2-4 weeks</td>
<td>90%</td>
<td>25-60%</td>
</tr>
<tr>
<td>3-6 months</td>
<td>100%</td>
<td>90-100%</td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth</td>
<td>99.8%</td>
<td>100%</td>
</tr>
<tr>
<td>1-6 months</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

## Testing Infants for HIV

| Maternal HIV status unknown | • Serologic testing on mother or infant  
|                           | • If positive then virologic testing of infant |
| HIV-infected mother on ART (infant at low risk) | • Virologic testing at 14-21 days, 1-2 months, and 4-6 months  
|                                              | • Repeat a positive result |
| HIV-infected mother not on ART (infant at high risk) | • Virologic testing at birth, 14-21 days, 1-2 months, and 4-6 months  
|                                              | • Test 2-4 weeks after cessation of ART if previous results negative  
|                                              | • Repeat a positive result |
| End points | • Two positive virologic tests  
|           | • Two negative virologic tests (≥1 month & ≥4 months)  
|           | • Some advocate for antibody testing at 12 and 18 months to confirm absence of maternal antibodies |

Case Study

• A 32 yo female at 39 weeks gestations is admitted for induction of labor due to GDM. Her male partner of 12 years is HIV+ and on ART with an undetectable viral load. The patient reported that her last sexual contact with the partner was at the time of conception. She has tested negative for HIV about 8 months prior and 1 month prior to admission. A rapid HIV test result was pending at the time of the consultation call. The patient stated that she was not having acute HIV symptoms.

• Should the induction of labor be delayed to allow for a definitive rule-out of HIV in the mother?
Case Study Resolution

• HIV risk is very low, even if the mother was sexually active with her HIV+ partner during pregnancy

• As her partner is virologically suppressed, the risk of sexual transmission is low

• Since the induction is non-emergent, it is reasonable to perform HIV viral load testing to definitively rule out acute HIV infection and to delay the induction until results are obtained

• However, if the mother is very clear she has had no risk behavior during the window period, the induction could continue as scheduled
Summary

• pH and fern tests are inaccurate methods of identifying PROM

• Fetal fibronectin has limited utility in the prediction of preterm birth

• FLM tests are excellent predictors of lung maturity but lung maturity is not an indicator of an infant’s readiness for post-natal life

• HIV screening algorithms for pregnant women are identical to those used for non-pregnant individuals; infants born to HIV-positive mothers must undergo virologic testing