Retrospective Assessment of Prenatal Alcohol Exposure by Detection of Phosphatidylethanol in Stored Dried Blood Spots

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Alcohol is a Teratogen

- Alcohol passes easily through the placenta from the mother's bloodstream into her baby's blood.
- The blood alcohol level (BAC) of the fetus rapidly becomes close to or equal to the BAC of the mother\(^1\).

\(^1\text{Am J Obstet Gynecol 112: 387-393.}\)
Alcohol is a Teratogen

- Ethanol elimination is impaired in the fetus due to reduced metabolic capacity and reuptake of amniotic fluid containing ethanol further prolongs fetal exposure time.

- Despite awareness initiatives, 1 in 13 pregnant women report drinking alcohol in the past 30 days\(^1\).

\(^1\)CDC, MMWR 61(28):534-538.
The Effect of Alcohol on Fetal Development

Periods of Fetal Development

- **Central Nervous System**
  - Weeks 3 to Full Term
- **Ears**
  - Weeks 4¼ to 20
- **Teeth**
  - Weeks 6¼ to Full Term
- **Palate**
  - Weeks 6¾ to 16
- **External Genitalia**
  - Weeks 7 to Full Term
- **Lower Limbs**
  - Weeks 4½ to 9
- **Upper Limbs**
  - Weeks 4½ to 9
Fetal Alcohol Spectrum Disorders

- Fetal alcohol spectrum disorder (FASD) is an umbrella term describing the range of conditions that can occur in a person whose mother drank alcohol during pregnancy.

<table>
<thead>
<tr>
<th>Abnormal facial features</th>
<th>Difficulty in school (especially with math)</th>
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<tbody>
<tr>
<td>Small head size</td>
<td>Learning disabilities</td>
</tr>
<tr>
<td>Shorter-than-average height</td>
<td>Speech and language delays</td>
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<tr>
<td>Low body weight</td>
<td>Intellectual disability or low IQ</td>
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<td>Poor coordination</td>
<td>Poor reasoning and judgment skills</td>
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<tr>
<td>Hyperactive behavior</td>
<td>Sleep and sucking problems as a baby</td>
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<tr>
<td>Difficulty with attention</td>
<td>Vision or hearing problems</td>
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<tr>
<td>Poor memory</td>
<td>Problems with the heart, kidneys, or bones</td>
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</table>
Diagnosing FASD can be hard because there is no medical test for it.

To diagnose FASD, doctors look for:

- Abnormal facial features
- Lower than average weight and/or height
- Central nervous system problems including small head size, problems with attention and hyperactivity and poor coordination
- Evidence of prenatal alcohol exposure
Early Diagnosis and Treatment of FASD

- Effective interventions and treatments are heavily reliant on proper diagnosis at an early age (under 3 years of age), at which time the nervous system problems are typically not yet apparent.

- Many children affected by fetal alcohol exposure do not have these distinctive facial characteristics, making confirmation of maternal drinking during pregnancy or detection of prenatal alcohol exposure essential for diagnosis.
Detection of Prenatal Alcohol Exposure

- Self-report methods of maternal drinking history during pregnancy can be unreliable due to recall bias as well as fear of stigmatization and embarrassment.

- Detection of Phosphatidylethanol (PEth) has been shown to be an indicator of moderate to heavy alcohol intake, at levels that could put a fetus at risk for potential alcohol-induced birth defects.

- Our laboratory has developed and validated a method for extracting and detecting PEth from dried blood spot samples on Guthrie cards from neonatal heel sticks.
Phosphatidylethanol (PEth)

- Phosphatidylethanolols (PEth) are a group of phospholipids with a common phosphoethanol head group onto which 2 fatty acid chains are attached.
• PEth can form in red blood cells as a component of the cellular membrane.
• PEth is a direct alcohol biomarker, meaning that ethanol is incorporated into the final product.
Phosphatidylcholine (PC)

Phosphatidic acid and choline

Phospholipase D (H₂O)

Phosphatidic acid and choline

Red Blood Cell Membrane (Phospholipids)

Red Blood Cell
Phosphatidylcholine (PC)

Phosphatidylethanol (PEth)

Phospholipase D (Ethanol)

Phosphatidylethanol (PEth)
Detection of PEth in dried blood spots

- Filter paper cards lyse and fix red blood cells completely and prevent further PEth synthesis or degradation.

- The stability of PEth in dried blood spots means minimal sample degradation during storage at RT, 4°C, or -20°C.
Degradation of PEth Over 12 months at RT

% of initial PEth concentration

MONTHS STORED AT ROOM TEMPERATURE

* p < 0.001
Detection of PEth in dried blood spots

- PEth is extracted from the dried blood spots into methanol and detected using an Agilent 6460 liquid chromatography-tandem mass spectrometry (LC-MS-MS) system.

- Our laboratory uses a very sensitive method to detect low concentrations of PEth from blood spot samples, with a limit of detection of 2 ng/mL.
Prevalence of prenatal alcohol exposure?

- The Behavioral Risk Factor Surveillance System (BRFSS) sponsored by the CDC found that from 2006 to 2010 the prevalence estimate for any alcohol use in the past 30 days was 7.6% among pregnant women.

- The prevalence estimates for binge drinking in the past 30 days was 1.4% among pregnant women.

- These prevalence estimates are based on self-report surveys.

- One prevalence study at the University of New Mexico found that universal screening of de-identified DBS cards at a university hospital revealed that 15.5% were positive for PEth (Bakhireva LNS. Alcoholism, clinical and experimental research. 2013;37(6):1008-15).
Project Aims and Methods

AIMS
1. Analyze the frequency of dried blood spot specimens that are positive for PEth.
2. Determine the prevalence rate of prenatal alcohol exposure in the general newborn population of Iowa.
3. Demonstrate the efficacy of detecting PEth levels from residual, stored dried blood spot cards using the PEth assay system developed at our laboratory.

METHODS
1. Collect 250 anonymous residual blood spot specimens from the Iowa repository.
2. Elute PEth from three 3.1 mm blood spot punches in methanol, separate by HPLC, and identify and quantify level of PEth using mass spectrometry.
Concentration of PEth Measured in Residual DBS

- 4% positive
- 8.2 ng/mL to 30.1 ng/mL
- Average - 17.6 ng/mL

10 newborns (4%) above the limit of quantitation (PEth ≥ 8ng/mL)
23 newborns (9%) above the limit of detection (PEth ≥ 2 ng/mL)
217 newborns (87%) below the limit of detection (PEth < 2 ng/mL)
University of New Mexico Hospital
15.5% positive PEth
20 – 337.5 ng/mL
Long-Term Goals

- Determining prevalence rates of babies exposed to dangerous levels of alcohol in utero is important to:
  - Identify vulnerable populations at a state level
  - Target prevention and treatment resources
  - Evaluate the strengths and limitations of prevention, intervention, and treatment strategies.
  - Determine if there is a need for universal screening of prenatal alcohol exposure to identify babies at risk for FASD.

- The ultimate goal is to provide objective prevalence estimates of prenatal alcohol exposure taken from a large, general-population sampling of newborns from multiple states to establish the scope and seriousness of this public health issue.
Thank You!

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