Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Progress Reports
PowerPoint Slides

June 2008

Table of Contents

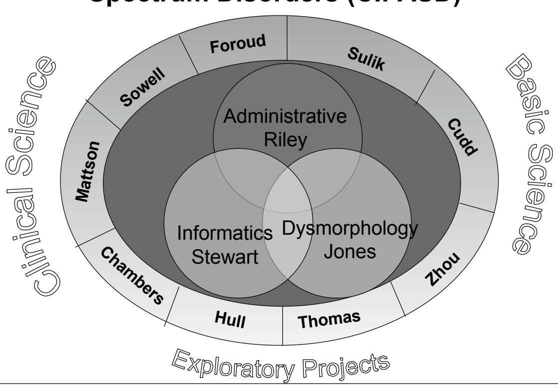
Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Administrative Core
Dr. Ed Riley

Administrative & Scientific Leadership

- CIFASD projects
  - Cores: Administrative, Dysmorphology & Informatics
  - Clinical Components: Chambers, Foroud, Mattson and Sowell
  - Basic Science Components: Cudd, Sulik & Zhou
  - Developmental projects: Thomas and Hull
    - Recruitment of new scientists to the Consortium
- Committees
  - Tissue Banking
  - Alcohol Use Questionnaire

Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)



CIFASD Liaison

- Administrative Core
- Steering Committees
- Science Advisory Board

- Assists in setting priorities for the Informatics Core

Communication Facilitation

- Conference calls (monthly)
  - Principal Investigators
  - Other project key personnel
  - Science Advisory Board
  - NIAAA advisors
- NIAAA calls and emails
- CIFASD.org website
- Biannual meetings
- Presentations

Conference Calls

- Monthly Calls for both Basic Science and Clinical Groups to monitor project progress and encourage project interaction and collaboration
  - Coordinated participant schedules
  - Determined dates and times
  - Sent notification and reminder emails
  - Served as moderator on the calls and set the agenda
  - Uploaded mp3 recordings of calls to website
- Working Group Calls (as needed)
  - Mattson, Chambers, Goodlett and Foroud

Science Advisory Board

- Dr. James West
  - Past Vice President for Research, Texas A&M Health Sciences Center
- Dr. Martin Teicher
  - Director of the Developmental Biopsychiatry Research Program, McLean Hospital
- Dr. Daniel Savage
  - Regent's Professor and Chair, Department of Neurosciences, University of New Mexico
- Dr. Kimberly Espy
  - Associate Vice Chancellor for Research, University of Nebraska, Lincoln
- Dr. Faye Calhoun
  - Past Deputy Director of NIAAA

CIFSAD.org Website

- Updated contact information
  - Existing and new members
- Updated latest news and upcoming events
- Updated publications
- Uploaded progress reports
- Added group email links page
- eJungle liaison for site additions, updates and monthly maintenance

Meetings

- January 2008 Rockville, MD
  - Basic Science January 10th and 11th
  - Clinical Group January 17th and 18th
  - Prepared annual progress report and uploaded PDF and individual PPTs to website
  - Evaluated projects and set goals for upcoming year
- June 2008 Rockville, MD
  - Separate Basic Science and Clinical days
  - Joint CIFASD meeting
  - Joint CIFASD & PASS project meeting
  - Uploaded PPTs to website and prepared PDFs of PPT slides

Meeting Preparation

- Coordinated participant calendars and selected dates and locations
- Contracted group rates for sleeping rooms at the selected hotel
- Assisted in reserving meeting space
- Arranged for airfare and other transportation for the PI, Science Advisory Board, Scientific Director and invited guests
- Prepared and distributed meeting materials and the meeting agendas
- Invited outside experts to the meetings to present their research findings on FASD

Outside Experts

- Barbara Finlay
  - Cornell University
- Cynthia Bearer
  - Case Western Reserve
- Alexandre Medina
  - Virginia Commonwealth University
- William Guido
  - Virginia Commonwealth University

Meetings, Presentations & Posters

- “Initiatives of Mother and Child Health Care: Strategies and International Partnership”. September, 2007: Rivne, Ukraine
- “Interventions and Treatment for Alcohol-Affected Individuals: The Next Challenge” by Marcus Institute. October, 2007: Atlanta, GA
- Virginia Commonwealth University. November, 2007: Richmond, VA
- National Organization on Fetal Alcohol Syndrome. November 2007: Washington, D.C.
- Alcohol Research Group. November 2007: Emeryville, CA
- SAMHSA Fetal Alcohol Spectrum Disorders Center for Excellence. December 2007: Rockville, MD
- NorCal Society of Toxicology Meeting. April 2008: San Francisco, CA
- “Consequences for Children Affected by Maternal Drug & Alcohol Usage: A Multi-Disciplinary Approach” by Parents for Children. March 2008: London, UK
- Alcoholism and Stress: A framework for future treatment strategies. May 2008: Volterra, Italy

Thank you

USGA U.S. OPEN 2008
TORREY PINES GOLF COURSE SAN DIEGO, CALIFORNIA JUNE 9 - 15, 2008

Informatics Core Report
Collaborative Initiative on Fetal Alcohol Spectrum Disorders

Andrew D. Arenson
aarenson@iupui.edu

11 June 2008

INDIANA UNIVERSITY

Outline

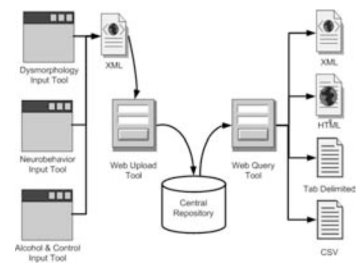
- Any new data?
- Overview of how Informatics gets done.
- What's been done?
- What's coming next?
- What about Basic Science?

Data in the CIFASD Central Repository

Cumulative Data Submitted	APS	ESL	KJK	KJR	LRB	PMP	PMS	PMR	PMK	SMS	SMM	SMF	SJS	SJD	TCM	Total
Dysmorphology	-	-	73	69	94	57	165	427	-	119	143	201	326	-	-	1,674
Neurobehavior	-	-	-	-	-	84	113	-	-	105	35	138	135	-	-	610
3D Facial Imaging	-	-	-	-	55	-	-	-	-	48	-	107 +2	163 +36	-	-	373 +38
Alcohol & Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Records as of March 31, 2008. Numbers following '+' reflect changes since December 31, 2007

How the software fits together



The data dictionaries define the variables used everywhere.

Data Dictionary example

Neurobehavioral Phase II Data Dictionary Beta

Field	Label/Question	DataType	ValueRange	Units	Default	Required?	Description	Comments
Element - Subject Information								
VerStatus	VerStatus	Integer	0 - unverified; 1 - verified		0	YES		Only verified records can be inserted into central repository
GlobalID	Subject ID	Text				YES	Subject's unique identification number	The GlobalID will differ depending on the site. The GlobalID can be a combination of letters, numbers and dashes, starting with a valid three letter code representing one of the projects in the CIFASD consortium.
DOB	Subject Date of Birth	Medium date	1/1/1970 to 12/31/3000			YES	Subject's date of birth	
Sex	Subject Sex	Integer	1 - Male; 2 - Female			YES	Subject's sex - either male (M) or female (F)	
Hand	Subject Handedness	Integer	0 - Right; 1 - Left; 2 - Mixed Right; 3 - Mixed Left;			YES	Subject's dominant hand - either right (R), left (L), mixed-right administered as dominant hand (MR), or mixed-left administed as dominant hand (ML)	A subject may be considered mixed, but a note should be made on which hand was used more often, and therefore was used as the dominant hand for such elements as Grooved Pegboard
Ethnicity		Integer	1 - Hispanic or Latino; 2 - No: of Hispanic or Latino; 3 - Unknown			YES	Hispanic or Latino(1), Not Hispanic or Latino(2), Unknown(3)	

Image by Yelena Yezerets

What's been done?

	Data Dictionary	Access DB Input Tool	XML Export	Web Submission	Web Report
Dysmorphology	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Neurobehavior	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Neurobehavior II	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3D Facial Imaging	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
EE Alcohol & Control	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Core Alcohol &	[illegible]				
Follow-up/Outcome	[illegible]	[illegible]			
Ultrasound	[illegible]	[illegible]			
Infant Neurobehavior	[illegible]	[illegible]			

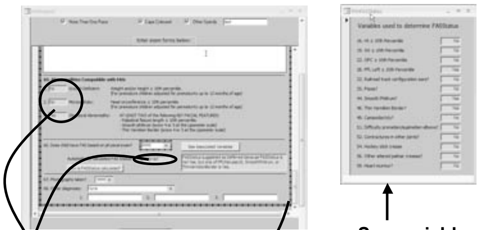
- ***Bold/Italics*** refers to change in status or significant work since 1 September, 2007.
- Infant Neurobehavior refers to Bayley and Maternal Questionairre variables only (not heart rate monitoring).

Data entry during exam: Pilot

- ***Secure, Mobile computer***
  - Data encrypted at all times.
  - Data easily backed up to USB thumb drive.
- ***Updates to Input Tool***
  - Seventeen changes, including: 9999s for all fields; Right Palpebral Fissure Length, Other Diagnosis, and age calculations.

Initial Results:
- Scribe needed – examiner can't also enter data.
- Stylus-based entry not helpful.
- Interface improvements requested …

Data entry during exam: Next test



- **Automatic calculations**
- **Visual status**
- **See variables without scrolling**

Infant Neurobehavior – Bayley

Image by Yelena Yezerets

Infant Neurobehavior – Maternal Questionnaire

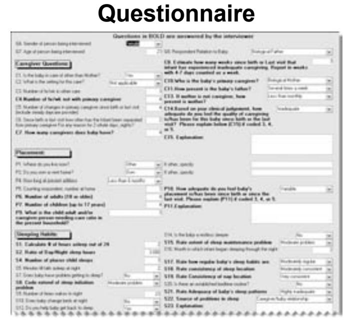


Image by Yelena Yezerets

Neurobehavior II – Subject

Image by Yelena Yezerets

Neurobehavior II – Import options

Image by Yelena Yezerets

Neurobehavior II – Vineland II Parent

Image by Yelena Yezerets

Neurobehavior II – WISC, verified

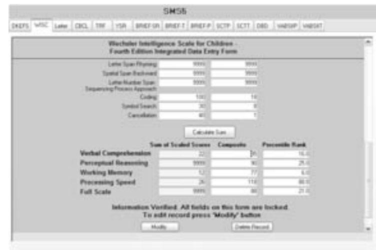


Image by Yelena Yezerets

Primary Record / Cross-Data Queries

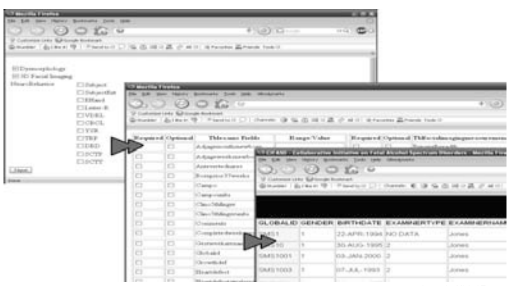


Image by Michel Tavares

What's coming next?

- Neurobehavior phase II – finish upload/query
  - Testing by users needed
- Dysmorphology during exams – finish input/upload/query
  - Testing by users needed
- Dysmorphology primary record & Cross-dataset queries
  - Testing by users needed
- Expanded Alcohol & Control – finish upload/query
  - Testing by users needed
- Core Alcohol & Control – Data Dictionary, etc.
  - Comparison to EEAC variables needed

What's coming next? (cont)

- Infant Neurobehavior (Bayley/Maternal) – Upload/query
  - Testing by users needed
- 3D Facial Imaging – Store in-progress files.
  - Reconsider in light of AVL changes
- EEAC and Followup-Outcome – Add nutritional vars.
  - Variables and where to include them needed
- Ultrasound – Update for biophysical profiling vars.
  - Variables and where to include them needed
- Infant Neurobehavior – Add heart rate monitoring vars.
  - Variables and where to include them needed

- ***Order of Priorities needs to be set.***

What about Basic Science?

- Available to all, including Basic Science projects:
  - Archive data via the IU Massive Data Storage System.
  - Use SPSS and SAS on IU's central systems.
  - Share data via the IU Slashtmp service for sending files and IU's Oncourse for a repository of files.
- Basic Science Repository? Can we establish requirements?

June 3, 2008

Acknowledgements

- The work presented here was done by the CIFASD Informatics Core:
  - Craig Stewart, PI
  - Bill Barnett, Senior Investigator
  - Andy Arenson, Project Manager
  - Michel Tavares, Lead Developer
  - Yelena Yezerets, Developer
  - Manju Pruthviraj, Developer

INDIANA UNIVERSITY

Dysmorphology Core – U24 AA014815 (Jones, PI)

Progress Report PowerPoint presentation not submitted.

Choline Availability and FASD

Jennifer Thomas
Developmental Project

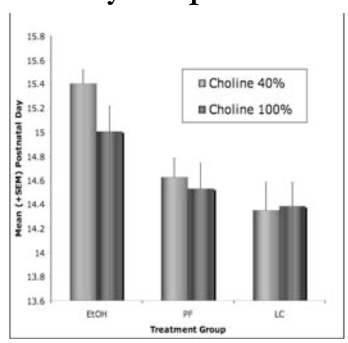
Does choline supplementation during the 3rd trimester mitigate ethanol's teratogenic effects?

- Previously shown that choline supplementation from PD 2-21 can reduce the severity of working memory deficits following prenatal alcohol exposure
- Currently collecting data:
  - Subjects are exposed to 6.0 g/kg/day ethanol from GD 5-20 and 5.25 g/kg/day on PD 2-9
  - Subjects receive various doses of choline chloride from PD 2-9

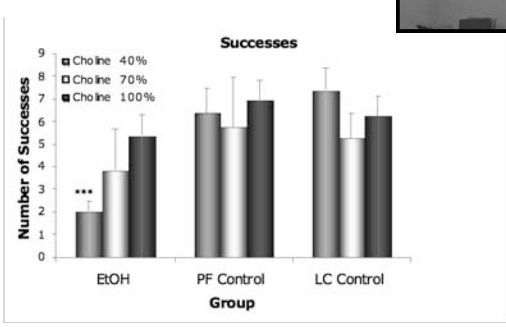
Does choline deficiency exacerbate ethanol's teratogenic effects?

Sprague-Dawley rats were randomly assigned to:	100% Choline	70% Choline	40% Choline
EtOH (6.0 g/kg/day) GD 5-20			
Pair-fed Control (PF)			
Ad lib Chow Control (LC)			

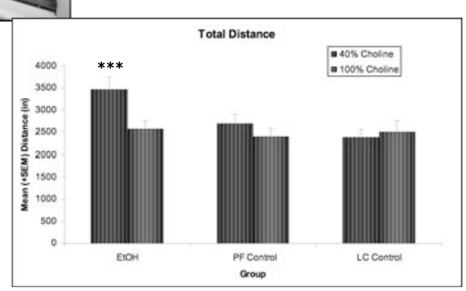
Eyes Opened



Hindlimb Coordination

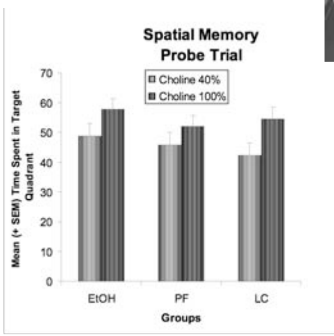


Open Field Activity

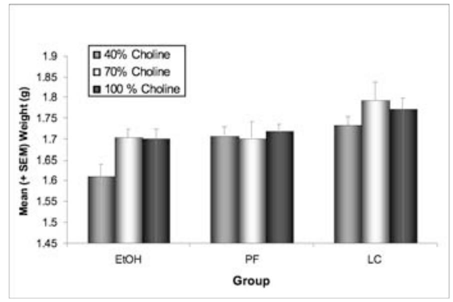


1

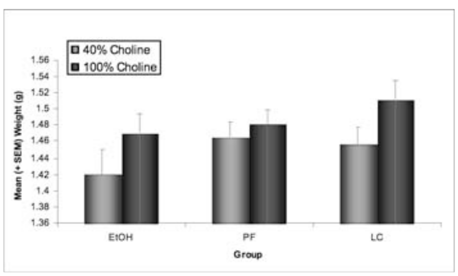
Morris Water Maze



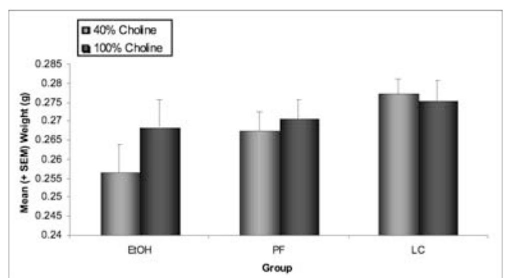
Whole Brain PD 30



Forebrain Weight PD 50



Cerebellum Weight PD 50



Summary

For many physical and behavioral measures, the combination of prenatal alcohol and choline deficiency produces the most severe alterations

Does ethanol induce choline deficiency?

Carl Keen and Jan Uri-Adams are currently examining levels of choline and metabolites following prenatal alcohol exposure

Evaluation of Early Markers of Prenatal Alcohol Exposure

An Update
May 2008

Andrew D Hull MD

Rationale for Study

- **Most FASD cases identified late because of failure to meet milestones**
- **Early detection of FASD allows early intervention**
- **Earlier interventions – more effective**
- **Prenatal detection would allow earliest interventions possible**

Previous Prenatal Studies

- **Limited number of studies:**
  - **Alcohol related impairment of frontal cortex and cerebellar development** (Wass, 2001)
  - **Alcohol related poor head growth and cerebellar diameter** (Handmaker, 2006)

Aims

- **To develop and evaluate ultrasound markers of prenatal alcohol exposure**
- **To compare these markers in 2nd and 3rd trimester scans**

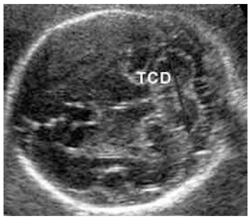
Methods

- **Prospective pilot study- 2004 - Ongoing**
- **Collaborative Initiative on Fetal Alcohol Spectrum Disorder Consortium**
- **Ukraine 2 sites**
- **6,745 pregnant women screened by TWEAK and AUDIT Questionnaires**
- **Inclusion criteria:**
  - **Gestational age: 10-40 wks**
  - **Alcohol exposed group (1-2 drinks x 10 per month)**
  - **Control group**

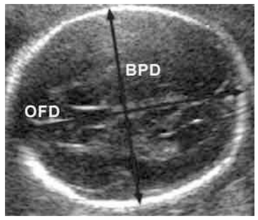
Sonography

- **Routine serial ultrasound exams**
- **Studies at ~24 & ~34 wks compared**
- **Routine biometry**
- **Specific brain measurements**

Brain Measurements

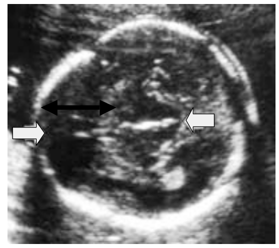


Transverse Cerebellar Diameter (TCD)



Occipital Frontal Diameter (OFD)

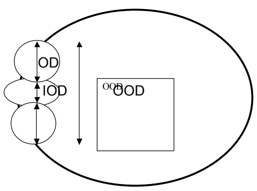
Brain measurements



Caval Calvarial Distance (CCD)

Frontothalamic Distance (FTD)

Orbital Measurements



- Outer Orbital Diameter (OOD)
- Intra Orbital Diameter (IOD)
- Orbital Diameter (OD)

Results

N	2nd Trimester	3rd Trimester
Alcohol-exposed	84	47
Control	82	31
Total	166	78

Maternal Demographics

Characteristic	Exposed (n=84)	Controls (n=82)	p-value
Maternal age	26.2 ± 5.7	24.7 ± 4.1	NS
Marital Status: Single (%)	10.7	1.2	0.017
Low Socio-economic Status (%)	51.2	31.7	0.006
Vitamin Use (%)	64.3	89.0	0.001
Smoking (%)	50.6	2.5	0.001

Alcohol Consumption Pattern Among Alcohol-Exposed & Comparison Subjects (Signs of Risk Drinking)

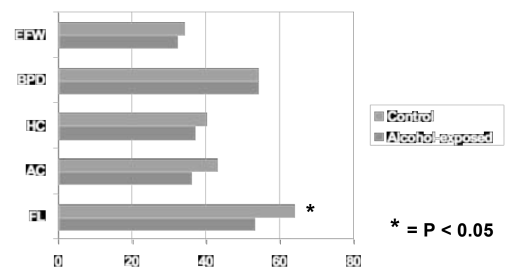
Signs of Risk Drinking	Alcohol Exposed	No Alcohol	p-value
	%	%	
Tolerance ≥ 6	67.1	1.4	<0.001
AUDIT ≥ 6	27.4	0.0	<0.001
TWEAK ≥ 6	69.6	1.4	<0.001

* AUDIT & TWEAK are alcohol screening questionnaires

Alcohol Consumption Pattern Among Alcohol-Exposed & Comparison Subjects (Absolute Ounces of Alcohol)

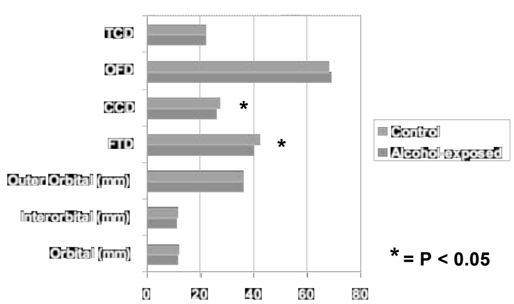
Absolute Ounces of Alcohol (AA)	Alcohol Exposed	No Alcohol	p-value
	Mean±s.d	Mean±s.d	
Periconceptional period:			
AA per day	1.07±1.4	0.02±0.2	<0.001
AA per drinking day	5.01±4.6	2.96±4.8	0.399
Pregnancy:			
AA per day	0.14±0.3	0.0004±0.002	<0.001
AA per drinking day	2.23 ±3.6	0.20±0.00	0.442

2nd Trimester



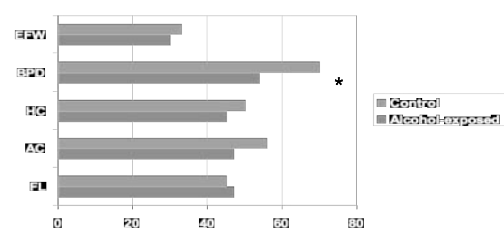
* = P < 0.05

2nd Trimester



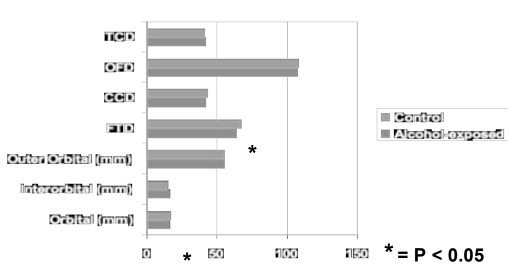
* = P < 0.05

3nd Trimester



* = P < 0.05

3rd Trimester



* = P < 0.05

Conclusions

- **Alcohol exposed fetuses have:**
  - **Reduced frontothalamic distance in both 2nd and 3rd trimesters**
  - **Reduced caval calvarial distance in 2nd trimester**
  - **Reduced BPD and orbital diameter in 3rd trimester**

Correlation of US findings with Dysmorphology data

Pregnant Women:

- **Alcohol-exposed (n=84)**
  moderate/heavy alcohol exposure
- **Comparison (n=82)**
  no/minimal alcohol exposure

Newborn Children:

- **FASD (3 FAS & 18 Deferred)**
- **No FASD (n=122)**

Statistical Analysis

- Prenatal ultrasound measures compared among:
  - FASD vs. no FASD
- Fetal growth measures expressed as gestational age-specific percentiles
- ANOVA, ANCOVA (adjustment for smoking, vitamin use)

Fetal Growth Measures (2nd trimester) FASD vs. No FASD

Fetal Growth Measures*	FASD (n=21)	No FASD (n=121)	p-value
	Mean±sd	Mean±sd	
Estimated fetal wt %	27.6±4.2	35.0±1.8	0.105
Biparietal diameter %	47.0±7.1	56.1±2.9	0.236
Head circumference %	28.5±4.4	41.9±1.8	0.006
Abdominal circumference %	27.2±7.6	43.3±3.2	0.053
Femur length %	47.0±5.4	62.5±2.2	0.010

* All models adjusted for smoking

Fetal Growth Measures (3rd trimester) FASD vs. No FASD

Fetal Growth Measures*	FASD (n=21)	No FASD (n=120)	p-value
	Mean±sd	Mean±sd	
Estimated fetal wt %	24.2±4.0	33.4±2.3	0.054
Biparietal diameter %	48.9±7.0	66.7±4.2	0.033
Head circumference %	33.9±5.6	53.8±3.3	0.003
Abdominal circumference %	47.3±6.3	54.2±3.5	0.350
Femur length %	30.9±6.3	51.9±3.6	0.005

* All models adjusted for smoking

Fetal Brain Measures (3rd trimester) FASD vs. No FASD

Brain Measure	FASD (N=21)	No FASD (N=120)	p-value*
	Mean±s.e.	Mean±s.e.	
Transverse Cerebella Diameter (mm)	40.8±0.4	41.9±0.5	0.178
Occipital Frontal Diameter (mm)	107.4±2.5	107.8±1.5	0.888
Caval-Calvarial Distance (mm)	41.6±1.0	43.3±0.6	0.127
Frontothalamic Distance (mm)	63.9±1.2	66.3±0.7	0.080
Outer Orbital Diameter (mm)	54.0±0.9	55.5±0.6	0.180
Interorbital Distance (mm)	14.9±0.6	15.6±0.3	0.301
Orbital Diameter (mm)	16.8±0.4	16.9±0.3	0.880

* Adjusted for gestational age & smoking in pregnancy

Reminder

- Previously demonstrated significant differences between alcohol exposure groups on selected somatic and brain growth measures in second and third trimester ultrasounds
  - Femur length percentile (2nd trimester only)
  - Caval-calvarial distance (2nd trimester only)
  - BPD percentile
  - Frontothalamic distance
  - Orbital distance (3rd trimester only)

Summary

- Significant differences on selected somatic and brain growth measures on ultrasound between infants with some structural features of FAS and those without on newborn physical exam:
  - Head circumference percentile
  - BPD percentile (3rd trimester)
  - Abdominal circumference percentile (2nd trimester)
  - Femur length percentile
  - Frontothalamic distance (3rd trimester)

Conclusion

- First demonstration on prenatal ultrasound that measures of growth and frontal brain are correlated with structural features of FASD on a newborn physical examination
- Specific Prenatal ultrasound measures may have some utility in early identification of fetuses with FASD

Next Steps

- **Further correlation of prenatal ultrasound findings with postnatal assessment of:**
  - **Dysmorphology**
  - **CNS imaging**
  - **Neurodevelopment**
- **Evaluation of predictive value of morphologic sonographic markers**

Next Steps (2)

- **Addition of Measures of Fetal Behavior**
- **Correlation of Morphology with Fetal Behavior Measures**
- **Development of screening triage tool combining best set of measures at specific gestational ages**
- **Test tool in practice**

Measures of Fetal Behavior

- Biophysical Profile
- Spontaneous "startles"
- Evoked startles

Biophysical Profile (BPP)

Component	Notes	Score
Fetal Movement	≥ 3 body or limb movements	0/2
Fetal Tone	One active flexion/extension of limb or opening and closing of hand	0/2
Fetal Breathing Movements	≥ 1 episode (incl hiccups)	0/2
Amniotic Fluid Volume	2x2 cm pocket	0/2

BPP Technique

- Record elements as part of routine scanning
- Do for each subject at 18-26 and >26 week scans
- Maximum time for BPP 30 minutes
- Record time taken for 8/8 or total score and elements completed by 30 mins

BPP (normal circumstances)

- Periodicity of most fetal activity varies according to fetal state
- 1F (quiet sleep) average time to 8/8 26mins
- 2F (REM) 3-5 mins
- 4F (active state) 3-5 mins
- Usually do BPP for up to 30 mins

BPP (normal circumstances)

- 97.5% 8/8 – normal
- 1.7% 6/8 – equivocal
- 0.52% 4/8 – abnormal
- 0.18% 2/8 – abnormal
- 0.06% 0/8 - abnormal

Utility of BPP

- No randomized trials
- Original study by Manning almost 80% reduction in perinatal mortality
- Perinatal Mortality Associated with BPP scores
  - 8/8 - 1.86/1000 *
  - 6/8 - 9.76/1000
  - 4/8 - 26.3/1000
  - 2/8 – 94/1000
  - 0/8 – 285.7/1000
- * If BPP 8/8 0.8/1000 within 7 days

Alcohol and BPP

- No studies addressing effects in humans
- If give Moms at 37 weeks a couple of glasses of wine and measure BPP - breathing movements cease but other movement unchanged
- In sheep chronically fed alcohol – the suppressive effect on breathing disappears with multiple exposures

What will be effects in humans?

- Don't know!

Startle Responses

- Protocol – observe fetus during routine imaging at <14, 18-26 & >26 weeks
- Record spontaneous startles (30 min)
- Generalized sudden movement starting in limbs lasting 1 second
- After observation period wait for 2 minutes of inactivity then:
- Attempt evoked startle (after 14 wks only) - 2sec FAS – if startle response within 4.5 sec +ve, if not -ve

Natural History of Startles

- Spontaneous
  - Start at 8 weeks
  - Decrease in frequency with increasing gestational age
- Evoked
  - Can evoke a response from 24 weeks

Alcohol and Startles

- Spontaneous
  - Seems to increase number of spontaneous startles at all gestational ages
- Evoked
  - Seems to reduce likelihood for an evoked startle

BPP & Startles

- Never been looked at together
- Never been looked at systematically
- Never been correlated with anatomy
- Never been correlated with outcome

Research Plan

- Continue to accrue cases
- Total of 300 subjects (including previous subjects)
- 75 per arm per year 2 years
- Morphology and brain measurements as before
- Add in fetal neurobehavioral measures at <14, 18-26 & >26 weeks

Research Plan (2)

- Physical exam for all newborns
  - FAS, not FAS, deferred – local ped
  - FAS, not FAS, deferred – dysmorph
  - Deferred reexamined later – and FASD diagnosis based on further exam…..
- Bayley at 6/12 and 12/12

What next?

- Evaluate ultrasound data further – structural abnormalities
- Collaborations within Consortium
  - Sulik project - Mice vs Man – Abstract submitted to Smith meeting. Explore similarities / differences between mouse data and findings in humans.
  - Cudd project – Sheep vs Man – Feasibility?
  - Coles project
    - BPP, startles, habituation and postnatal response – correlations between fetal and postnatal behavior

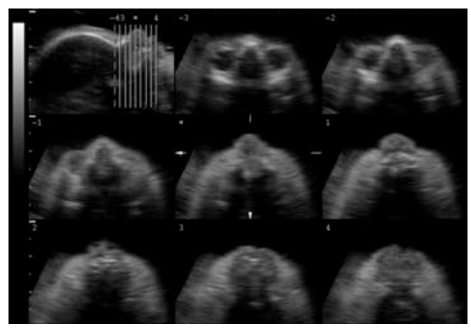
New Ultrasound Projects 1

- Effects of alcohol on development of eye
  - Prenatal measurements of orbital dimensions already in place – additional laterality measurements being recorded
  - Add In 3DUS?
  - Add in postnatal assessment of globe dimensions and structure of eye
    - Already have interest and expertise in Ukraine
    - Need specialized pediatric ophthalmic transducer
    - Level of interest from consortium?

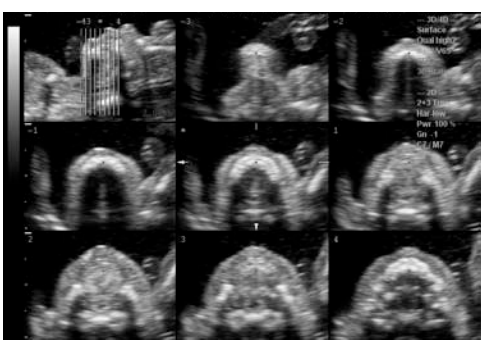
New Ultrasound Projects 2

- Effects of alcohol on dentition / facial skeleton
- Equipment in place and expertise developing to allow imaging of fetal facial skeleton using 3D/4DUS
- Can assess facial skeleton using various modalities including 3D rendering, multiplanar and multislice
- Level of interest from Consortium?

Multislice Orbits to Maxilla



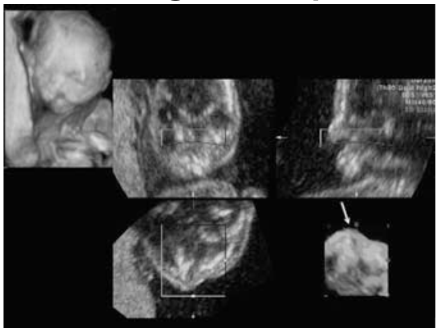
Multislice Maxilla to Mandible



New Ultrasound Projects 3

- Prenatal vs Postnatal dysmorphology
- Equipment in place and expertise developing to allow imaging of fetal face using 3D/4DUS
- Potential for developing sonographic measures of facial structures
- Potential for comparing US and examination
- Level of interest from Consortium?

Rendering & Multiplanar



Thank You

Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Translational Studies of FASD Using a Sheep Model

UO1 AA017120

May 2008 Progress Report

Currently, we are breeding adult ewes to produce the lambs that will serve as the subject population for the ovine component of the CIFASD project.

Several ewes have already been bred, randomly assigned to treatment group, treated (as appropriate for that group), and have given birth to lambs.

The first facial measurements (from a longitudinal series of three) have been obtained from all CIFASD lambs born thus far.

Eyeblink classical conditioning will begin at 9 weeks age, T-maze training will begin at 14 weeks age.

None of the lambs produced as part of the CIFASD project has yet reached the age at which behavioral training is scheduled to begin.

Specific Aim 1 Hypothesis:
1st-trimester and 3-trimester alcohol exposure models will cause:
- Facial Dysmorphology,
- Reductions in Brain Volume,
- More severe effects following 3-trimester exposure

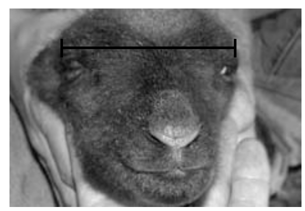
Facial Dysmorphology-
Proposed: Obtain standardized measures of facial features, at three timepoints:
- at birth,
- at two months (weaning),
- at five months

Progress thus far: We have procured calipers (spreading and sliding) for measurement of facial features, and have begun obtaining facial measurements of newborn lambs

Reduced Brain Volumes-
Proposed: Obtain 3-D structural MRI at 3 months age

Progress thus far : No lambs have yet reached 3 months age

Standardized Facial Measurements:



Width at medial fronto-temporal junction

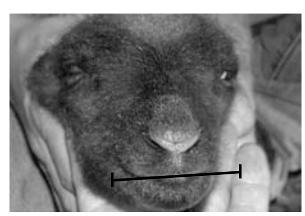
1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Width of head across right and left tragus

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:



Width of lower face across line extended through corners of mouth to outer edges of face

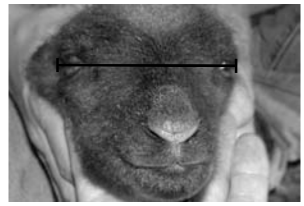
1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Inner canthal width

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

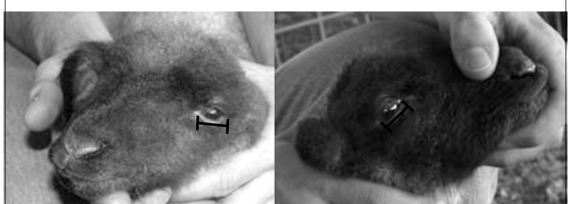
Standardized Facial Measurements:



Outer canthal width

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:



Palpebral fissure width

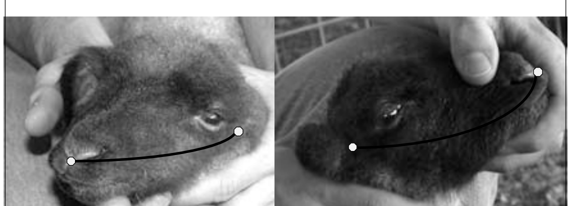
1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Tragus (left and right) to nasion

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:



Tragus (left and right) to upper philtrum

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Length of mandible;
tragus (left and right) to mandibular symphosis

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Midpoint between inner canthi to midpoint between nostrils

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Midpoint between inner canthi to midpoint between nostrils

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Philtrum length

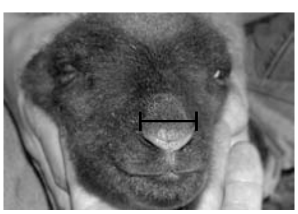
1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:

Tip of nose to mandibular symphosis

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Standardized Facial Measurements:



Nasal width at lateral border

1 Minimal Frontal Width
2 Bizygomatic Width (N/A)
3 Bitragal Width
4 Bigonial Width
5 Inner Canthal Width
6 Outer Canthal Width
7 Palpebral Fissure Width
8 Upper Facial Depth
9 Midfacial Depth
10 Lower Facial Depth
11 Nasal Length
12 Nasal Bridge Length
13 Philtrum Length
14 Lower Facial Height
15 Total Facial Height (N/A)
16 Ear Length
17 Nasal Width

Specific Aim 2 Hypothesis:

1st-trimester and 3-trimester alcohol exposure models will cause:
Cerebellar-dependent learning and memory deficits,
Hippocampal-dependent learning and memory,
More severe deficits following 3-trimester exposure

Proposed: Begin eyeblink classical conditioning (150 trials / day) at 9 weeks age
delay eyeblink conditioning to assess cerebellar-dependent learning,
trace eyeblink conditioning to assess learning that requires both cerebellar and hippocampal function
8 consecutive days, 15 ten-trial blocks / day,
9 CS-US paired trials & 1 CS-alone "probe" trial / block

Begin T-maze training at 14 weeks age
to assess frontal cortex-related executive function and
hippocampal-dependent spatial working memory
spatial alternation
pseudo-random alternation of reinforced goal-box
3 trial-couplets / day (1 "forced-run" / 1 "choice"),
4 consecutive weeks (M-F),
position discrimination / reversal
1 goal-box consistently reinforced, reinforced side switched after week 1
3 "choice" trials / day, 2 consecutive weeks (M-F)

Progress thus far: We have obtained normative data from untreated lambs for use in establishing T-maze and eyeblink conditioning training parameters.
No lambs have yet reached behavioral testing age (≥ 9 wks).

Specific Aim 3 Hypothesis:

1st-trimester and 3-trimester alcohol exposure models will cause:

Significant neuronal loss in cerebellum, hippocampus, raphe nuclei

More severe cell loss following 3-trimester exposure

Proposed: Stereological cell counts of:

- **Cerebellar Purkinje cells,**
- **Hippocampal pyramidal neurons from CA1 and CA3,**
- **Dentate gyrus granule cells, and**
- **Serotonergic neurons from the raphe nuclei**

Progress thus far: No lambs have completed the behavioral testing phase; no brains have yet been harvested for cell-count processing

Specific Aim 4 Hypothesis:

Choline supplementation to pregnant ewes during gestation will ameliorate effects of 3-trimester alcohol exposure on behavioral and brain outcomes

Proposed: Supplement diet of pregnant ewes with choline during 3-trimester alcohol exposure period, quantify effects of alcohol exposure with methods similar to those of first 3 Specific Aims.

Progress thus far: The determination as to when to begin choline supplementation (immediately after mating, or at the beginning of the 2nd trimester), and in conjunction with which exposure model (1st-trimester or 3-trimester) will be informed by results from the rodent component of the CIFASD project; at this time there is no progress to report on Specific Aim 4.

Photolabeling of Alcohol Binding Sites on L1 – U01 AA014812 (Miller/Charness)

Progress Report PowerPoint presentation not submitted.

Magnetic Resonance and Diffusion Tensor Imaging of a Mouse FASD Model

The Sulik Laboratory
The University of North Carolina

CIFASD
Progress Report
June 2008

Specific Aim 1:

To utilize high resolution Magnetic Resonance Imaging (MRI) and Diffusion Tensor Imaging (DTI) as a high throughput screening platform to provide comprehensive documentation and discovery of the ethanol-induced CNS dysmorphology that results from prenatal ethanol exposure at embryonic and early fetal stages of development.

Emphasis to date has been directed toward this Aim.

Specific Aim 2:

To define, utilizing high resolution Magnetic Resonance Imaging (MRI) scans and 3-D reconstructions, the facial dysmorphology that results from prenatal ethanol exposure at embryonic and early fetal stages in mice and to relate the character and severity of these defects to accompanying brain abnormalities.

Specific Aim 3:

To identify, utilizing selected sections or 3-D reconstructions of MRI scans, regions other than the brain or face that may serve as diagnostic indicators of prenatal ethanol exposure.

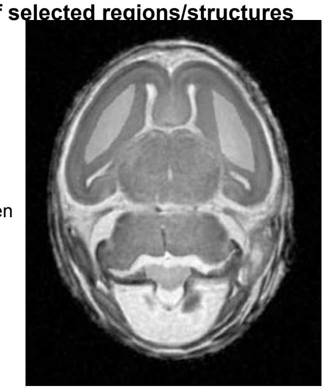
Study Design

Treatment group	Number of GD17 litters/fetuses: Control	Treated	Selection	Source
Acute IP (~450mg/dl)			Based on defects	Sulik Lab
GD 7	5 (7)* (4)*	5 (19) (3)		
GD 8	5 (6) (1)	5 (6)		
GD 9	5 (4) (1)	5 (5)		
GD 10	5 (6) (1)	5 (9)		
GD 11	5	5		
Acute Dietary (~200mg/dl)			Random	Sulik Lab
GD 7	5	10		
GD 8	5	10		
GD 9	5	10		
GD 10	5	10		
GD 11	5	10		
Chronic Dietary				
GD 7-11			Random	Zhou Lab
~200mg/dl	5	10		
~100mg/dl	5	10		
GD 12-16			Random	Sulik Lab
~200mg/dl	5	10		
~100mg/dl	5	10		
GD 7-16			Random	Zhou Lab
~200mg/dl	5	10		
~100mg/dl	5	10		
Totals	80	135		

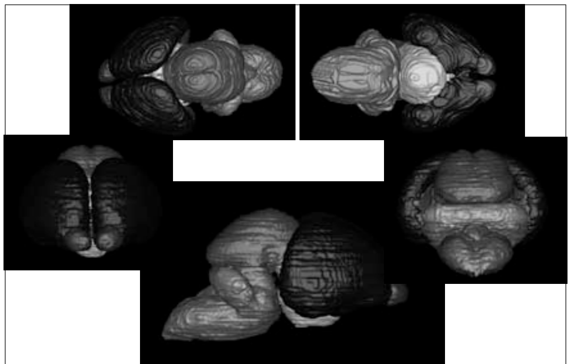
5/20/2008 *Numbers of brains that have been imaged for MRI *Numbers of brains that have been imaged for DTI

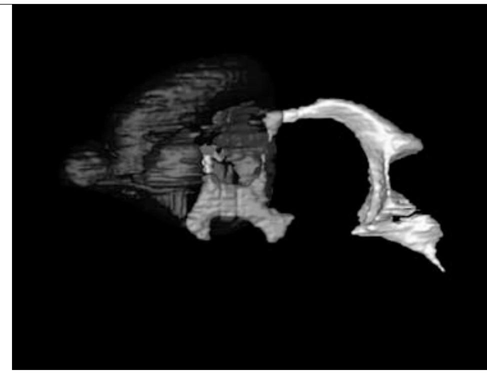
Segmentation of selected regions/structures

- R&L Olfactory bulb
- R&L Cortex
- Septal region
- Lateral Ventricles
- Third Ventricle
- Fourth Ventricle
- R&L Hippocampus
- R&L Caudate/Putamen
- Diencephalon
- Mesencephalon
- Pons + Medulla
- Cerebellum
- R&L globe (eye)
- R&L lens
- Pituitary

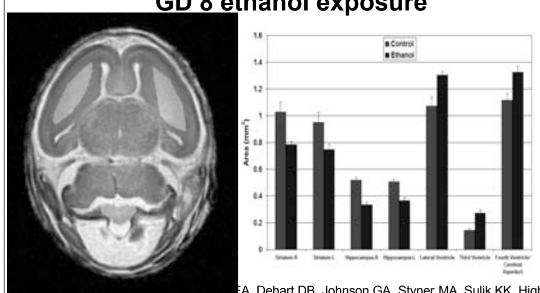


1



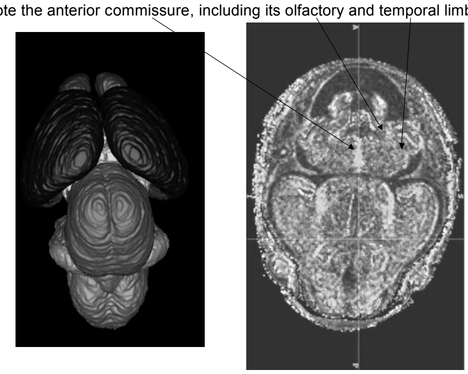


The hippocampus and striatum are deficient and the ventricles are enlarged following GD 8 ethanol exposure

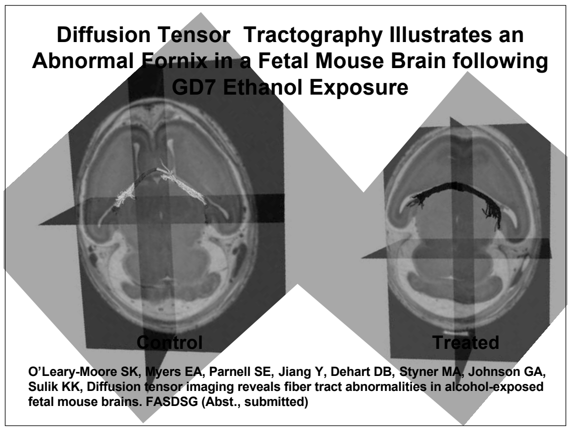


EA, Dehart DB, Johnson GA, Styner MA, Sulik KK, High resolution magnetic resonance imaging defines ethanol-induced brain abnormalities in fetal mice: effects of acute insult on gestational day 8. (Full paper, in preparation)

Diffusion Tensor Imaging @ 39μ illustrates fiber tracts.
Note the anterior commissure, including its olfactory and temporal limbs

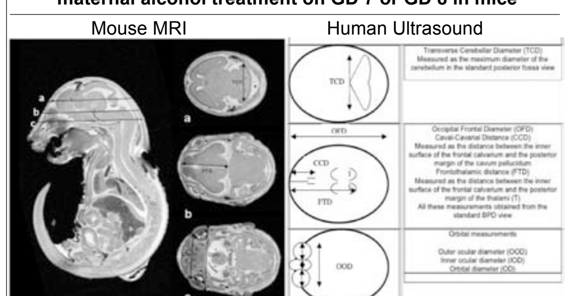


Diffusion Tensor Tractography Illustrates an Abnormal Fornix in a Fetal Mouse Brain following GD7 Ethanol Exposure



O'Leary-Moore SK, Myers EA, Parnell SE, Jiang Y, Dehart DB, Styner MA, Johnson GA, Sulik KK, Diffusion tensor imaging reveals fiber tract abnormalities in alcohol-exposed fetal mouse brains. FASDSG (Abst., submitted)

Linear measure comparisons show reduced frontothalamic distances in alcohol-exposed humans and following acute maternal alcohol treatment on GD 7 or GD 8 in mice



Sulik KK, O'Leary-Moore SK, Parnell SE, Myers EA, Dehart DB, Johnson GA, Chambers CD, and Hull AD, High resolution magnetic resonance imaging of alcohol-exposed fetal mice confirms and informs human prenatal ultrasound studies. Proc of the Greenwood Genetics Center (Abst., in press)

Gestational day 9 ethanol treatment does not result in significant changes in **linear** CNS measures. However, the **volume** of the midbrain and hindbrain is reduced.

Parnell SE, Johnson GA, Sulik KK, Brain abnormalities resulting from gestational day nine ethanol exposure in the mouse: a study utilizing high resolution MRI, Alcohol Clin Exp Res,2008 (Abst., in press)

Pilot studies conducted in collaboration with the Zhou laboratory have shown FAS-like craniofacial dysmorphology in GD 17 fetal mice following chronic maternal dietary alcohol exposure (peak maternal BAC approximately 200mg/dl) during days 7 through 16 of pregnancy in C57Bl/6J mice.

Imaging studies for this exposure paradigm will be initiated in the very near future.

Publications

Parnell SE, Johnson GA, Sulik KK, Brain abnormalities resulting from gestational day nine ethanol exposure in the mouse: a study utilizing high resolution MRI, Alcohol Clin Exp Res,2008 (Abst., in press)

Sulik KK, O'Leary-Moore SK, Parnell SE, Myers EA, Dehart DB, Johnson GA, Chambers CD, and Hull AD, High resolution magnetic resonance imaging of alcohol-exposed fetal mice confirms and informs human prenatal ultrasound studies. Proc of the Greenwood Genetics Center (Abst., in press)

O'Leary-Moore SK, Myers EA, Parnell SE, Jiang Y, Dehart DB, Styner MA, Johnson GA, Sulik KK, Diffusion tensor imaging reveals fiber tract abnormalities in alcohol-exposed fetal mouse brains. FASDSG (Abst., submitted)

Parnell SE, O'Leary-Moore SK, Myers EA, Dehart DB, Johnson GA, Styner MA, Sulik KK, High resolution magnetic resonance imaging defines ethanol-induced brain abnormalities in fetal mice: effects of acute insult on gestational day 8. (Full paper, in preparation)

FASD MRI/DTI Presentations

Oct 2, 2007 - NIAAA/INSERM meeting Rockville MD (K. Sulik)

Nov 16, 2007 - Lecture to UNC Pathology graduate students (K. Sulik)

Nov 19, 2007- Seminar to UNC Toxicology Department (L. Myers)

Dec 12, 2007 - Lecture to UNC medical students (K. Sulik)

Jan 8, 2008 - OB Gyn Grand Rounds, Charlotte, NC (K. Sulik)

Jan 15, 2008 - Lecture to UNC Ortho/Pedo graduate students (K. Sulik)

Feb 22, 2008 - Lecture to UNC Dental Hygiene students (K. Sulik)

March 5, 2008 - Lecture to Neurobiology Graduate Students (K. Sulik)

March 12, 2008 - Lecture to Neurodevelopment Post docs (K. Sulik)

April 10, 2008 - Invited talk for Northern California Society of Toxicology meeting (K. Sulik)

May 22, 2008 - UNC Pathology Grand Rounds (K. Sulik)

Debbie Dehart, Jian Dong, Kathy Sulik
Liz Myers, Shao-Yu Chen, Scott Parnell

Student assistants:
Jacob Ament
Amber Khan
Stephen Pecevich

Shonagh O'Leary-Moore UNC **Martin Styner UNC NDRC** **Al Johnson Duke U, CIVM**

3

Mouse Model Neuro-Facial Dysmorphology: Translational & Treatment Studies

Bruce Anthony
Yun Liang
Shiaofen Fang
Li Shen
Charles Goodlett
Feng C. Zhou

Specific Aims

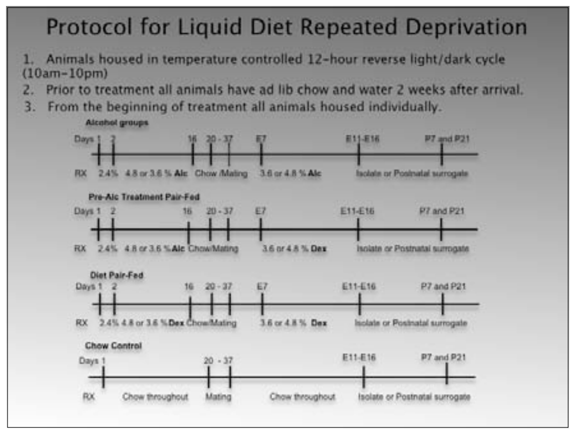
- **Aim 1. To advance the understanding of sources of variation in abnormal facial development induced by prenatal alcohol exposure as a function of the dose and developmental timing of alcohol exposure in a C57BL/6 mouse model.**
- **Aim 2. To determine longitudinally the extent of brain structural and neuro-facial abnormalities as a function of the dose and developmental stage of alcohol exposure.**
- **Aim 3. To determine the extent to which the Neurotrophic peptides NAP/SAL will provide long-term protection against alcohol-induced neuro-facial dysmorphology and neurobehavioral deficits.**

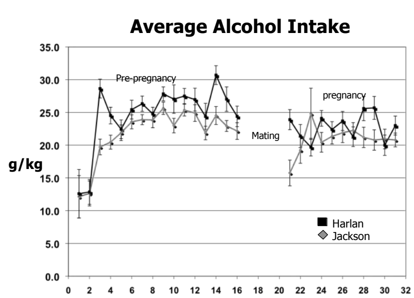
1. Mouse Model for Dose and Timing of Alcohol Exposure

Base line of C57BL6 mice lines from Halan and Jackson breeder used in Drs. Zhou and Sulik's laboratories

(a)Drinking level

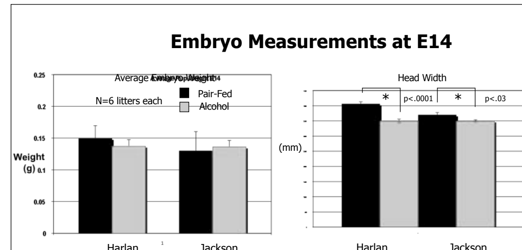
(b) Teratogenesis



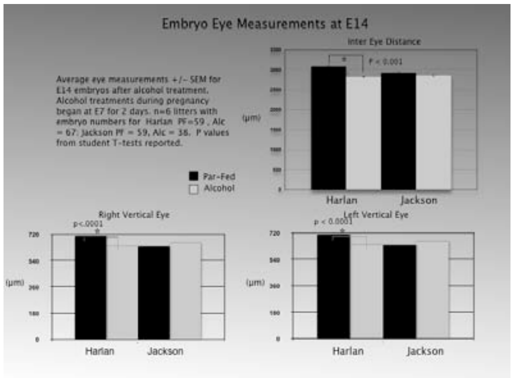


Averages in g/kg:
Pre-pregnancy: Harlan 24.6 +/- 1.6, Jackson 21.8 +/- 1.3
Pregnancy: Harlan 22.8 +/- 1.5, Jackson 20.6 +/- 1.6

Figure. Daily average alcohol intake comparing Jackson and Harlan C57Bl/6 mice. Values are reported as daily averages for each group +/- SEM. Pre-pregnancy: n= 46 Jackson, 46 Harlan; Pregnancy period n=15 Harlan. 20 Jackson

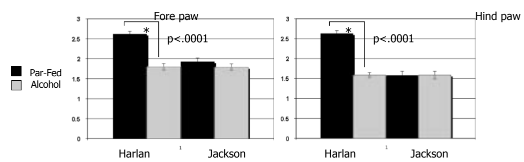


Average measurements +/- SEM of E14 embryos after alcohol treatment. Alcohol treatments during pregnancy began at E7 for 2 days. n= 6 litters with embryo numbers for Harlan PF=59 , Alc=67: Jackson PF=59, Alc=38. P values from student T-tests are reported.



Limb development

Paw Webbing for E14 Embryos



Webbing measurements +/- SEM for E14 embryos after alcohol treatment. Alcohol treatments during pregnancy began at E7 for 2 days. n=6 litters with embryo numbers for Harlan PF=59 , Alc = 67: Jackson PF = 59, Alc = 38. P values from student T-tests reported.

Jackson C57BL/6 E16 Embryo Defects -- Parnell and Sulik

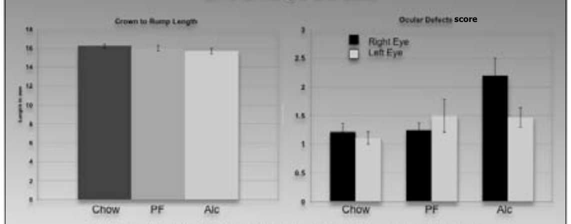


Figure. Measurement for E16 embryos after E7-E16 alcohol treatment. Ocular defects reflect increasing microphthalmia in a scoring system from 1 (normal) - 5 severe microphthalmia.

7

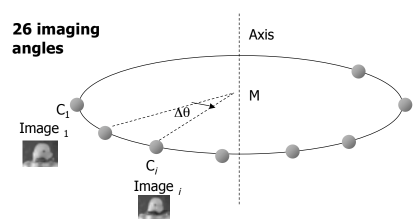
2D/3D Mouse Facial Image Analysis from Micro-Video Images

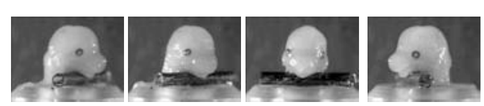
Shiaofen Fang, Li Shen, Ying Liu

Developing Two Approaches:

1. Multi-angle analysis: Carry out 2D image analysis from multiple angles, and combine the results to produce 3D features and more reliable classifications.
2. 3D reconstruction: Generate a 3D model from a sequence of 2D images, and carry our 3D image analysis directly on the 3D models.

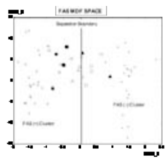
Image Data Collection





Multi-angle analysis

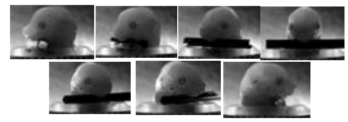
For each image angle, apply pixel based 2D image analysis.
- Principal Component analysis (PCA) for dimension reduction and feature selection
- Linear Discriminant Analysis (LDA) for pattern extraction and classification



Combine results from multiple angles
- Common features
  - Based on the image correspondence after morphing.
  - Weighted blending of common regions
- –Classifications
  - •by voting
  - •by combined probability

Preliminary test on multiple angle 2D analysis

- 14 subjects: 7 alcohol, 7 control
- Leave-one-out validation: picking one subject as test set from each group, and rotating 7 times.
- Analyses were done with 7 different angles.



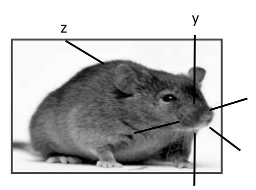
Results from 7 angles were combined based on (1) voting and (2) combined probability (same outcome occurred in this experiment).
A preliminary classification rate of 85.7% is evaluated
We are still working on ways to combine, blend, and visualize the 3D features.

	Prediction	
	FAS (+)	FAS(-)
FAS	7	0
Control	2	5

3D reconstruction: Improved algorithm

- Preliminary study shows that the original algorithm does not provide sufficient surface details necessary for the 3D analysis.
- We are currently working on an improved volume carving algorithm : Back project front voxels, and make decisions on their image correspondences based on a comparison of color values obtained form images of all angles.

Micro-CT Imaging of Craniofacial Skeletal Structure



Yun Liang, Huisi Ai, Bruce Anthony, Feng C Zhou

Study Objectives

- Identify a group of anthropometric geometries and bone features to differentiate subjects with alcohol exposure.
- Establish correlation between facial and skeletal features for FASD dysmorphology.
- Explore new means for diagnosis of craniofacial dysmorphology

Scan Procedure

Subjects: 16 controls and 12 alcohol treated mice.

MicroCT Scans: longitudinal scans at postnatal day 7 (P7) and P21.

Anesthesia Technique: Initiated in aninduction chamber with Isoflouranelevels @1.5 % and 0.8-1.2 liter/min, and a maintenance @0.5 liter/min during the 2-hour scans.

CT number Calibration: QA phantom with water, air, acrylic, bone (SB3) inserts

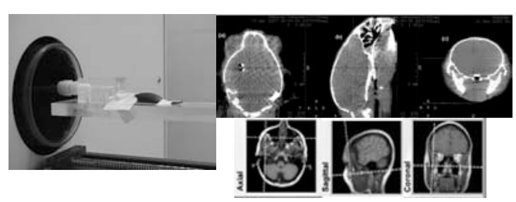


Image Analysis

•*Reconstruction of 3D skull image*:
skull tissues are segmented by a auto and global thresholding in CT density value followed by manual inspection of every image slice. Modification of auto segmentation is performed by manually redraw the skull surface boundaries at locations where there are mismatches between auto results and true anatomy;

•*3-D volume rendering :*
Volume rendering is generated using segmented skull tissues at the selected threshold value.

•*Length and volume measurement on bones:*
Volume and length of global and regional bones are performed using isosurface and skull volume data.

Image analysis tool: All image analysis is performed with software tool "MxView" (courtesy of Philips Medical Systems).

Reconstruction of 3D skull image

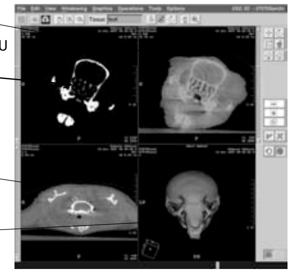
"MxView" tool

Display of an coronal slice

Segmentation result with a 1700-HU thereshold on the coronal slice

Display of an axial slice.

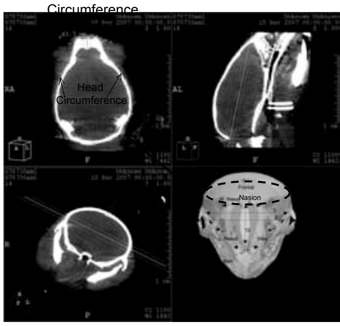
Skull volume rendering



Three thresholds, 800-HU (25% of SB3), 1300-HU(40% of SB3) and 1700 HU (55% of SB3)are used as "signature" thresholds for global segmentation to group bone into "soft", "medium", and "hard" categories. For facial surface, a threshold of 0-HU is applied.

Head Circumference Measurement

Circumference

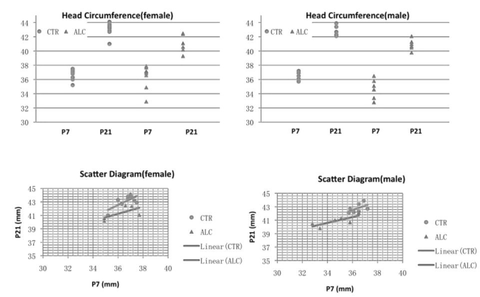


Mid-SagittalPlane of Head

Axial, coronal and sagittal planes are reformatted to align with mouse head.

The **Circumference**is extracted as the outer perimeter of skull enclosure in the axial plane at the level of "nasion". (the articulation of frontal and nasal bone)

Difference in Head Circumference
Control Vs. Alcohol Treated



A: Head circumference of ALC and Control measured at P7 and P21 days
B: The longitudinal trend of head circumference increases as mouse aged from P7 to P21.

CTR group has greater mean values for both genders . T-test is performed for statistic significance test.

Inter Orbital Bone Distance

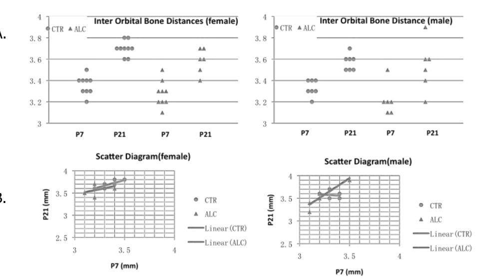
Facial surface can be rendered in MicroCT to assist finding the positions of mouse eyes.

Coronal planes are cut through the skull to extract the distance between orbital bones by finding the maximal distance between orbital bones.

Difference in Orbital Bone Distance
Control Vs. Alcohol Treated

A.

B.



A: Inter-orbital bone distance of ALC and Control measured at P7 and P21 days
B: The longitudinal trend of inter-orbital bone distance changes as mouse aged from P7 to P21.

CTR group on average has greater mean values for both genders. T-test is performed for statistic significance test.

Regional Bone Analysis

Control

Alcohol

Regional bone analysis (nasal bone shown as an example) shows features potentially interesting for alcohol related dysmorphology.

Landmarks and segmentation tools are used to extract anthropometric bone groups.

Summary of Progress

1. Established pan-pregnancy alcohol mouse model from either Harlen or Jackson C57BL/6 to study the timing of alcohol exposure relevant to human gestation.

1. Testing 2D and developing 3D analyses of mouse facial Microvideo imaging for comparative diagnosis of FASD facial dysmorphology between mouse and human.

2. Mouse skull bone is subject to change (e.g. Circumflex) upon mid-gestation alcohol exposure, which may underline the facial dysmorphology.

3. Established a model of longitudinal analysis of craniofacial bone growth after birth, to evaluate evolvement of craniofacial dysmorphology upon fetal alcohol exposure.

Spectrum of and Nutritional Risk Factors for FASD in Russia and Ukraine

Progress Report

September 1, 2007 - July 31, 2008

Investigators

- Tina Chambers - UCSD
- Ken Jones - UCSD
- Claire Coles - Emory
- Julie Kable - Emory
- Carl Keen - UC Davis
- Jan Uriu-Adams - UC Davis
- Andy Hull - UCSD
- Ludmila Bakhireva - UNM
- Lily Xu - UCSD
- Lubya Yevtushok
- Wladimir Wertelecki
- Lela Kavteladze
- Ludmila Joutchenko
- Anatoly Skalny
- Pavel Ogurtsov
- Irina Blinikova

Specific Aims

- To measure the birth prevalence and range of alcohol-related physical features and neurobehavioral impairment among children born to women who report consuming moderate to heavy amounts of alcohol in pregnancy relative to children born to mothers who report consuming low amounts or no alcohol during pregnancy
  - Evaluate alcohol quantity, frequency and timing in relation to growth, structure and neurobehavior
  - Assess infant development using early infancy measures of processing speed and attentional regulation skills and measures from the BSID II

Specific Aims

- To evaluate the contribution of maternal nutritional status of specific micronutrients to risk for various features of FASD including growth deficiency, structural features, and neurobehavioral impairment
  - Assess the relation of baseline nutritional status as measured in early pregnancy to alcohol exposure group
  - Evaluate the impact of micronutrient supplementation with or without choline on change in nutritional status from baseline to third trimester in alcohol-exposed vs. comparison pregnancies
  - Evaluate the predictive value of nutritional status for specific micronutrients with respect to alcohol exposure and specific pregnancy outcomes
  - Examine the relationship between indicators of oxidative stress and alcohol-related birth outcomes

Specific Aims

- To provide the performance site, local resources and human subjects for other current or proposed CIFASD projects.
  - 2-D ultrasound imaging (Hull)
  - 3-D facial imaging (Faroud)

Methods

- 2 sites: Rivne and Moscow
- Cohort study - exposed/unexposed
- Random assignment to multimicronutrient supplement with or without additional choline
- Dysmorphological exam, 2-D photographs
- Ultrasounds at Rivne site (Hull)
- BSID II at 6 months and 12 months at both sites; infant habituation/heart rate evaluation at 6 months included in Rivne site only
- 3-D facial imaging at Rivne site only (Faroud)

Accomplishments: Training and Process I

- Two training sessions (October, 07 and March 08) in Ukraine on new ultrasound measures, Bayley and Infant Heart Rate Monitoring with designated staff at performance site in Ukraine
- Training of new data manager in Ukraine (March 08)
- Two-week training of Russian lab technician on choline and vitamin measures in Dr. Keen's lab (April 08)
- IRB approvals in US and foreign sites
- Translation of study materials
- Finalization of all data entry tools
- EEAC ready for upload testing

Accomplishments: Training and Process II

- Selection and purchase of dose/form of 'registered' choline supplement available in both Ukraine and Russia - 700 mg. day
- Setup of arrangements for shipping of Ukraine samples to UC Davis
- Validation of physical exam data by Ken Jones for children previously evaluated by neonatologists/geneticists

Accomplishments: Data Collection

- Screening initiated in Ukraine, and re-initiated in Russia - May, 2008 - screening of 800 pregnant women per month, enrollment tracking on 40 per month
- Mineral analyses performed on previously collected blood samples in Russia (Keen presentation) - confirms significantly lower average zinc levels at first and third trimester in alcohol exposed group relative to un-or-low exposed group

Minerals in Binge-Drinking Women vs. Unexposed - Russia

	1st/2nd trimester N = 20			3rd trimester N = 20		
Mineral	Alc+	Alc-	P-value	Alc+	Alc-	P-value
Ca	88.5	93.8	0.35	88.8	96.8	0.19
Cu	2.2	2.4	0.41	2.0	2.6	0.04
Fe	1.4	1.1	0.35	1.0	1.2	0.55
Mg	18.1	19.0	0.49	17.8	19.0	0.31
Se	0.11	0.13	0.17	0.11	0.14	0.15
Zn	0.59	0.73	0.08	0.62	0.78	0.01

Accomplishments: Collaboration with Other Projects

- Dose selection for choline supplements reviewed with Thomas, Goodlett, Cudd
- Neurobehavioral and ultrasound measures compared with Cudd sheep model
- Ultrasound measures compared with Sulik mouse model
- Preparation for 3D facial imaging - site setup and plan for transmission of images (Rogers) and requirements for customs approval determined
- Development of exposure assessment for retrospective data with Mattson, Faroud

Accomplishments: Presentations/Publications

- UCSD Pediatric Grand Rounds - Chambers - Feb, 2008
- Abstract submitted by Sulik Group - with Chambers/Hull - to 2008 DW Smith Workshop
- 2 abstracts on ultrasound and alcohol consumption data accepted at First Central and Eastern European Summit on Preconception Health and Prevention of Birth Defects - August, 2008
- 1 abstract on alcohol consumption data accepted at American Public Health Association Meeting - October, 2008
- Ultrasound manuscript in press

Plans for July, 2008 - Jan, 2009

- Establish baseline choline levels with first round of sampling - August 2008 - and compare to Thomas results
- Negotiate 3D camera import to Ukraine - September 2008 and image children from pilot project
- Interface with PASS project (biomarkers and ultrasound) and Zeisel's UNC project (choline supplementation in pregnancy and infant neurobehavior)

Facial Imaging Project – U01 AA014809 (Foroud, PI)

Progress Report PowerPoint presentation not submitted.

A Multisite Neurobehavioral Assessment of FASD

Sarah Mattson, PI
CIFASD Clinical Group PI Meeting
June 11-12, 2008

Key Personnel

- Colleen Adnams, Co-PI, subcontract
- Claire D. Coles, PI, subcontract
- Julie A. Kable, Co-I, subcontract
- Wendy Kalberg, Co-PI, subcontract
- Philip A. May, PI, subcontract
- Edward P. Riley, Co-PI
- Elizabeth R. Sowell, PI, subcontract

Objectives

- To determine whether a neurobehavioral phenotype exists in children with fetal alcohol syndrome
- Whether the same phenotype exists in children with FASD who lack facial dysmorphology
- Whether the phenotype can be used for differential diagnosis
- Secondary aims, involving collaboration with other CIFASD projects and cores, are to determine the relationship between brain dysmorphology, facial dysmorphology, and neurobehavioral function.

Methods

- A standard neurobehavioral protocol will be administered to four groups of children at six sites
  - executive function, working memory, verbal function, and psychological symptomatology.
- In addition to children with FASD and non-exposed controls, children with low IQ scores or ADHD will be included as contrast samples.
- Using this heterogeneous sample and multivariate statistical methods, neurobehavioral profile specific to FASD will be sought.
- participants will also be assessed using methodology prescribed by the Dysmorphology Core and the facial and brain imaging projects of the CIFASD. Data from three broad domains (neurobehavior, dysmorphology, and brain morphology and function) will be analyzed both separately and together to address the main aim of the CIFASD: improving the diagnostic criteria for FASD.

Relationships with Other Projects/Investigators

- Brain Imaging
  - San Diego, Los Angeles, South Africa
- Facial Imaging
  - San Diego, Los Angeles, Atlanta, South Africa
- Dysmorphology
  - All sites

Participating Sites

- Center for Behavioral Teratology, San Diego State University, San Diego, CA
- Marcus Institute, a Division of Kennedy-Krieger Institute at Emory University, Atlanta, GA
- University of New Mexico, Albuquerque, NM
- Seven Northern Plains communities, including six Indian reservations
- University of Cape Town, South Africa
- the University of California, Los Angeles, Los Angeles, CA

Proposed Sample Sizes

Site	FAS/D	CON	IQ	ADHD
San Diego	50	50	25	25
Los Angeles	25-35	25-35	0	25
Atlanta	25-50	25-50	25	25
New Mexico	80	80	40	80
N. Plains	80	80	0	80
S. Africa	100	100	40	0
Total	360	360	130	235

Progress in First Funding Year September 1, 2007-July 31, 2008

- Conference Calls. Monthly conference calls have been held with site PIs and key Personnel.
- IRB Approvals. Submitted applications for IRB approval for the neuropsychological, dysmorphology, brain imaging, and 3D imaging portions of the study at each site. Approvals granted at all sites.
- Subcontracts. Two of three subcontracts have been fully executed. The Emory subcontract is awaiting final signatures of Emory officials.

Progress in First Funding Year

- Hiring. We have in place staff necessary to conduct the study, including a psychometrist, recruiter, and research associates/assistants. The subcontract sites have personnel in place, with the exception of Atlanta and South Africa which are waiting for funds to be released to hire personnel.
- Purchasing. We have purchased materials and equipment necessary to begin data collection at each site. Materials are being distributed.

Progress in First Funding Year

- Final Test Battery. Finalized the test battery taking into consideration reviewer and site-specific concerns.
- Material Development. Created working drafts of our test administration materials and scoring materials.
- Database Development. Worked with the informatics core to develop the input tool for Phase II. Received and piloted the beta version of this input tool and are actively working to move it towards its final form.

Progress in First Funding Year

- Training. Two training sessions were conducted in December 2007 and January 2008. All sites were present for one or both training sessions, which included data collection, scoring, data entry, input tool, and reliability procedures.
- Pilot Testing. UCLA, UNM, and SDSU sites have submitted pilot tapes for review. Administration procedures were approved.
- Data Collection. UNM has begun data collection at the Plains and UNM sites, UCLA and SDSU have subjects scheduled.

Progress in First Funding Year

- **Website**. Created group website using google groups to facilitate communication and transmission of relevant information.
- **Equipment**. Provided each site with video cameras and dvd recorders to archive data collection sessions and provide source for reliability assessment.
- **Alcohol Screening Questionnaire**. Created abstraction form for alcohol and other exposure data, and other demographic data. Provided to Informatics core for data base development.
- **Prepared NIH Progress Report**.

Progress in First Funding Year

- **Submitted Papers Using CIFASD Phase I Data**
  - May et al. Neuropsychological characteristics of Italian children with fetal alcohol spectrum disorders.
  - May et al. Neuropsychological study of FASD in a sample of American Indian children: The effects of processing simple versus complex information.

Progress in First Funding Year

- **Papers in Preparation Using CIFASD Phase I Data**
  - May et al. The effects of processing simple and complex information in children with fetal alcohol spectrum disorders.
  - Mattson, SN, Roesch, SC, Riley, EP, Adnams, C, Autti-Rämo, I, Fagerlund, Å, Kalberg, W., Korkman, M., May, PA, and the CIFASD. Neurobehavioral profile of children with heavy prenatal alcohol exposure.
  - Mattson, SN, Riley, EP, Autti-Rämo, I, May, PA, Konovalova, V., Jones, KL., Roesch, SC, and the CIFASD. Spatial learning and navigation deficits in an international sample of children with heavy prenatal alcohol exposure.

Progress in First Funding Year

- **RSA 2008 Posters Using CIFASD Phase I Data**
  - Kang, N, Vaurio, L, Doughty, RS, Riley, Mattson, SN. Objective measurement of activity levels in children with heavy prenatal alcohol exposure
  - Vaurio, L, Kang, N, Wagner, Riley, EP, Mattson, SN. Laboratory validation of parent-reported measures of inattention and hyperactivity in children with heavy prenatal alcohol exposure

Next Steps...

- Initiate data collection at all sites.
  - Neuropsych, facial imaging, brain imaging, dysmorphology
- Finalize Phase II neuro database and begin entering data.
- Enter existing data into new alcohol/demographic input tool when available.

Mapping the Brain the Face and Neurocognitive Function in FASD (U01)

Elizabeth R. Sowell, Ph.D.
UCLA

Progress as of June 2008

U01 Specific Aims

Specific Aim 1: To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on brain morphology and function. We will study differences in the patterns of results that occur across populations where drinking patterns may vary by making FASD/control comparisons within sites, and comparing the results across sites.

Specific Aim 2: To evaluate relationships between brain dysmorphology and facial dysmorphology both cross-sectionally and longitudinally to improve diagnostic criteria using facial morphology data from the dysmorphology core (cross-sectional data only) and the 3D camera project.

Specific Aim 3: To determine whether the anatomical "phenotype" relates to neurobehavioral profiles in children with fetal alcohol syndrome or FASDs.

Specific Aim 4: To investigate dysmorphology in the brains of human children based on findings in the mouse and sheep models conducted in the laboratories of Drs. Sulik, Zhou and Cudd.

Continued Progress from Previous Funding Period

Since our last progress report presented in January 2008, we have continued to analyze data collected by the brain imaging core. Our paper on white matter abnormalities and neurobehavioral correlates has now been published in the *Journal of Neuroscience* (Sowell et. al., 2008). A new manuscript on the impact of prenatal alcohol exposure on brain activation during verbal working memory has been submitted (O'Hare et al., Submitted). We have analyzed data for visuospatial working memory and language processing, and are preparing manuscripts with these results.

All of these studies directly address Specific Aim 1 in the new U01 project:

> **Specific Aim 1:** To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on brain morphology and function. We will study differences in the patterns of results that occur across populations where drinking patterns may vary by making FASD/control comparisons within sites, and comparing the results across sites.

Published Manuscripts:
Sowell E.R., Johnson A., Kan E., Lu, L.H., Van Horn, J.D., Toga, A.W., O'Connor, M.J., and Bookheimer S.Y., (2008) Mapping White Matter Integrity and Neurobehavioral Correlates in Children with Fetal Alcohol Spectrum Disorders. *Journal of Neuroscience*, 28(6):1313-9.

Submitted Manuscripts:
O'Hare E.D., Lu L.H., Houston S.M, Bookheimer S.Y., Mattson S.N., O'Connor M.J., and Sowell E.R. Altered frontal-parietal functioning during verbal working memory in children and adolescents with heavy prenatal alcohol exposure.

Abstracts:
O'Hare, ED, Lu, LH, Bookheimer, SY, McCourt, ST, Houston, SM, Mattson, SN, O'Connor, MJ, and **Sowell, ER.** (2007, November). Increased dorsal frontal and inferior parietal activation during verbal working memory among children and adolescents with prenatal alcohol exposure. Poster presented at the 37th Annual Meeting of the Society for Neuroscience, San Diego, California.

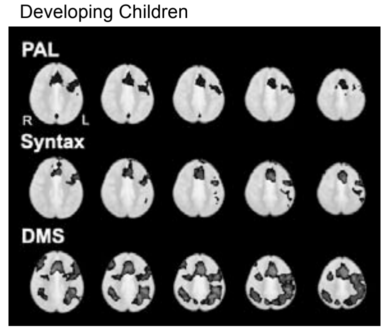
fMRI results from 3 different cognitive tasks in as many as 20 FASD children.

Paired Associates Learning (PAL) is a verbal paired associates learning task with auditory stimuli presentation (will be used in CIFASD continuation)

Synatx is a task which requires subjects to listen to pairs of sentences, and decide if the meaning is the same.

Delayed Match to Sample (DMS) is a verbal working memory paradigm in which subjects are required to memorize lists of letters.

Group Average Activation Patterns for Normally Developing Children

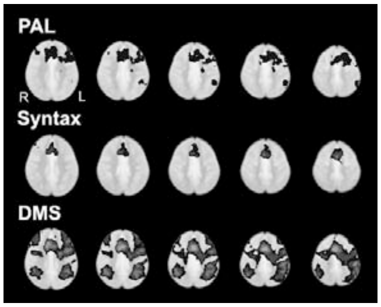


N = 16
7 to 15 years

N = 20
7 to 15 years

N = 20
7 to 15 years

Group Average Activation Patterns for FASD Children

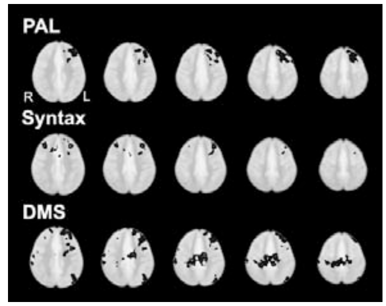


FASD N = 11
8 to 13 years

FASD N = 9
7 to 15 years

FASD N = 20
7 to 15 years

Brain Activation Increases in FASD Relative to Unexposed for Three fMRI Tasks

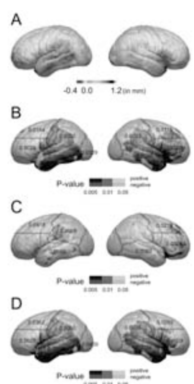


Con N = 16
7 to 15 years
FASD N = 11
8 to 13 years

Con N = 20
7 to 15 years
FASD N = 9
7 to 15 years

Con N = 20
7 to 15 years
FASD N = 20
7 to 15 years

Results suggest an over reliance on left anterior dorsal frontal cortices (for these verbal tasks) whether task demands are for learning and memory, working memory, or sentence comprehension. These dorsal frontal regions are relatively spared structurally in that these regions do not have cortical thickness abnormalities. Perhaps children with prenatal exposure have adapted these regions to compensate.

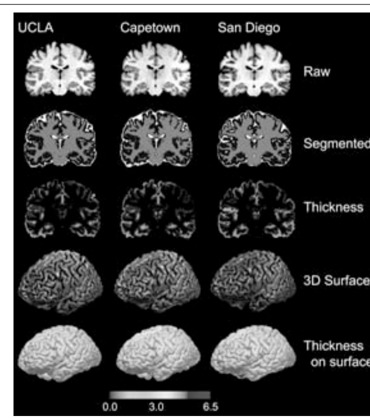


Progress since our last meeting in January has been primarily in 4 areas:

1) Selecting image acquisition protocols to accommodate all 3 data collection sites
2) Collecting pilot image data from the UCLA 3T Allegra system which is the same as the magnet in South Africa.
3) Collecting pilot data from the 3T magnet at UCSD.
4) Collecting Pilot data from the 3T magnet in South Africa.
5) Continued training for the neurobehavioral testing battery in collaboration with Sarah Mattson.

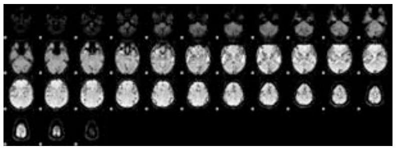
In February 2008, co-investigator Katherine Narr went to South Africa and met with Colleen Adnams and Ernesta Meintjies. The plans for that trip included (1) facilitating the translation of the functional imaging experiments from English to Afrikaans (done), (2) refining structural and functional imaging acquisition protocols to ensure compatibility with those used at the other imaging sites (done), (3) obtaining human phantom data with these imaging protocols at the South Africa site for later quantification of geometric distortions across all imaging sites (data collected), and (4) solving other logistical issues relevant to the successful acquisition of subject test data (ongoing process).

Same brain, three magnets.

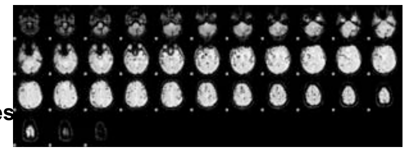


2

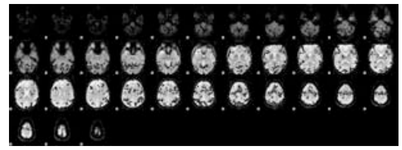
fMRI results PAL **South Africa**



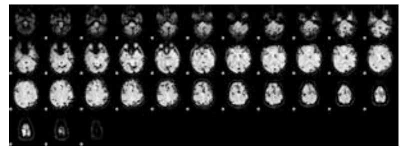
fMRI results PAL **Los Angeles**



fMRI results N-back **South Africa**



fMRI results N-back **Los Angeles**



Estimated start dates:

UCLA: First subject seen May 10 with 8 more scheduled for the summer.

South Africa: Projected start date August 2008. Note, population is already identified, so recruitment will not be a problem for catching up with delays in year 1.

San Diego: Projected start date June 2008.

Plans for the next funding period:

1) Data collection will commence or continue at all 3 sites.
2) Human phantom data will be analyzed for normalization of data across scanners.
3) Analyses of data collected in the earlier CIFASD will continue.