Use of a classification system for severity of neonatal sepsis to predict outcomes in premature infants

Allyson Abo*, Marta Barker, Naveed Hussain. Div. of Neonatology, Dept. of Pediatrics, Univ. of CT Health Center, Farmington, Connecticut.

Introduction: Despite advances in medical therapies, neonatal sepsis remains the leading cause of death and chronic organ damage in a neonatal intensive care unit (NICU). Severity of illness due to sepsis may have an influence on outcomes but at present no classification system is in use in neonatology. The **aim** of this study was to use a classification system for sepsis to help predict outcomes (survival, length of stay) in premature infants.

Methods: 1590 premature infants admitted to the NICU at the University of Connecticut Health Center between January 1999 and December 2003 were studied. Infants with nosocomial sepsis (onset > 3 days of life) were divided into four categories corresponding to their severity. Grade 1- confirmed sepsis without other organ dysfunction; Grade 2 - sepsis with transient hypotension, metabolic acidosis, or oliguria; Grade 3 - septic shock defined as need for pressors, or intravascular volume expansion; Grade 4 - multiple organ dysfunction defined as septic shock with at least one of the following: neurologic changes, seizures, disseminated intravascular coagulation, anuria, generalized edema, anasarca or thrombocytopenia. Neonatal outcomes studied were survival and length of stay in the NICU. The influence of gestational age, sex, race, and birth weight on primary outcomes were also studied. Data were analyzed using univariate and multivariate comparisons. **Results:** The overall incidence of nosocomial sepsis was 19% (n = 304). The incidence by grades was: Grade 1 (n=184, 60.5%); Grade 2 (n=27, 8.9%); Grade 3 (n=31, 10.2%); Grade 4 (n=62, 20.4%). The survival rates based on grades (1-4) of sepsis were: 97% (n, 179), 93% (n, 25), 81% (n, 25), and 81% (n,50), respectively. On univariate analyses, infants' sex, race, birth weight, or GA had no impact on sepsis-severity but survival and length of stay were significantly worse with increasing grades of sepsis ($p < 0.001$). On multiple logistic regression analyses, when corrected for race, sex, gestational age, and birth weight, the likelihood of infants' dying with grade 3 sepsis was 9.7 times that of grade 1 (95% CI 2, 40; $p = 0.0016$). Similarly, infants with grade 4 sepsis were 10.2 times more likely to die compared to those with grade 1 (95% CI 3, 33; $p = 0.0001$). However, in multivariate analyses for length of stay, the significance of grade of sepsis disappeared when either GA or birth weight were introduced as co-variates. **Conclusion:** Nosocomial sepsis is common (19%) in premature infants in the NICU. The proposed classification of sepsis into 4 grades was predictive of survival independent of gestational age and birth weight. **Significance:** A classification system for severity of sepsis would be useful in prognostication of survival and in formulation of research design for testing new therapies.

ABSTRACT

TITLE: Fetal Nasal Bone Hypoplasia is a Consistent Marker for Fetal Trisomy Between 16-21 Weeks Gestation

AUTHORS: Brotherton, Joy*; Cusick, William; Shevell, Tracy; Duchan, Lynn; Lupinacci, Cara.

INSTITUTION: Stamford Hospital, Department of Obstetrics and Gynecology, Stamford, CT, USA

OBJECTIVE: To assess the utility of fetal nasal bone hypoplasia in the detection of fetal aneuploidy between 16-21 weeks gestation.

METHODS: Prospective, cross sectional, ongoing study performed at a single institution between January 2003 to October 2004. At the time of amniocentesis between 16-21 weeks gestation, the fetal nasal bone length was measured as previously described. The nasal bone length for aneuploid fetuses was compared to previously established normative data.

RESULTS: A total of 380 amniocenteses for fetal karyotyping were performed. 13 cases of fetal aneuploidy were identified: Trisomy 21 (5), Trisomy 18 (3), and other (5). Nasal bone length was present in all fetuses and recorded in 12/13 cases. Fetal nasal bone hypoplasia ($<10^{th}$ percentile) was present in all cases of fetal trisomy and a single case of de novo translocation. The fetal nasal bone length averaged 0.55 multiples of the median (MoM) for trisomy 21 and 0.57 MoM for trisomy 18 fetuses.

CONCLUSIONS: Nasal bone hypoplasia is a consistent finding in trisomic fetuses between 16-21 weeks gestation. Assessment of fetal nasal bone length should be incorporated into the second trimester genetic sonogram. Additional data will help to better define the relative risk for fetal trisomy based on the fetal nasal bone length between 16-21 weeks gestation.

Effect of IL-6 and IL-11 on Oxidant Injury in Neonatal Rat Lung

Anne Chetty [1], Gong-Jee Cao[1], Nicholas Manzo [2], Heber C Nielsen [1] Aaron Waxman [2].
[1]Pediatrics, Floating Hospital for Children, Tufts-New England Medical Center, Boston, MA; and [2] Pulmonary Critical Care Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Significance: Oxygen, in addition to its beneficial role of minimizing the adverse effects of hypoxia, and its effect on growth factors, also has detrimental effect through production of reactive oxygen species. Interleukin 11 (IL-11) a cationic member of the IL-6 family has a wide range of effects on different cell types. IL-11, like many recently cloned growth factors, has pleiotropic effects on hematopoietic cells presumably depending on the cellular environment into which it is introduced. **Hypothesis:** Il-6 and IL-11 provide anti-inflammatory and cyto-protective effect during oxidant injury in neonatal lung. **Design**: Type II cells from 3 day-old neonatal rat pups were treated the cells with varying concentrations of either Il-6 or IL-11 (5-200ng/ml) at ~85% confluence for 24h prior to oxidant injury. The cells were exposed to 1mM H_2O_2 for 1h. The viability was assessed by tryptan blue dye exclusion and counted by hemocytometery. For in vivo experiments, 3 day old transgenic IL-11 overexpressing mouse pups (IL-11+) and wild-type pups were exposed to 95% oxygen or room air for three days. Tail clipping was done for genotyping. Lungs were removed and preserved in 3% formalin for paraffin embedding for histology and immunofluorescent studies. Immunohistochemistry for TUNEL analysis was performed using an in Situ cell death detection kit (Roche Applied Science) for both room air and hyperxia exposed pups (IL-11 + and wild type). The TUNEL positive fluorescent cells were counted in ten fields (200X) in each condition in all the four conditions. **Results**: Type II cells exposed to IL-6 and IL-11 prior to oxidant injury exhibited significantly improved survival compared to control cells control cells [60% survival in IL-6 pretreated cells compared to 50% in control, and 67% survival in IL-11 pretreated cells compared to 50 % in control $P<0.05$]. With in vivo hyperoxia exposure the number of TUNEL positive cells in hyperoxic pups was significantly increased compared to room air exposed animals [30% increase in hyperoxia-exposed pups compared to control $P < 0.05$]. The increase with hyperoxia was significantly reduced in the lungs from IL-11 + mouse pups [15% TUNEL positve cells in IL-11 (+) compared to 27 % in IL-11 (-) controls]. **Conclusion**:These studies demonstrate that IL-6 and IL-11 provide protective effects against oxidant-mediated injury in type II cells and in IL-11 transgenic mice in developing lung. **Funded By**: HL 67089, HL 074859, HL 37930

Preconception Uterine Cavity Length And The Risk Of Preterm Birth In Multifetal Pregnancy

Investigators: Allahna Coggins, MD*, Glenn Markenson, MD, Daniel Grow MD and Fadi Bsat MD

Baystate Medical Center Divisions of Maternal Fetal Medicine and Reproductive Endocrinology

Objective: To investigate the association of preconception uterine cavity length as a risk factor of preterm birth in multifetal pregnancies.

Study Design: All in-vitro fertilization (IVF) patients at our institution have a transabdominal ultrasound measurement of uterine cavity length performed prior to embryo transfer. We retrospectively reviewed those measurements obtained between January 2000 and September 2003 in IVF pregnancies that resulted in live multiple births from 20 to 37 weeks of gestation. All obstetrical charts were reviewed, and we included only patients whose preterm delivery was due to spontaneous preterm delivery in the absence of other obstetrical or medical complications. These pregnancies were divided into two groups: those who delivered after and before 34 weeks of gestation respectively. Unpaired t-test was then used to compare uterine lengths between the two groups.

Results: A total of 127 patients were included: 115 women delivered after 34 weeks of gestation compared to 24 who delivered before 34 weeks of gestation. The mean uterine depth was 76 mm (range = 53-104mm). In pregnancies delivered before 34 weeks gestation, the mean uterine cavity depth was 71 mm compared to 76 mm in patients delivered after 34 weeks ($p<0.01$).

Conclusion: Decreased uterine depth is associated with preterm birth in multiple gestations following IVF. Shorter uterine length may be another factor to consider upon the number of embryos transfer after IVF to decrease the risk of preterm birth.

ELECTRON MICROSCOPE EXAMINATION OF CERVICAL MICROSTRUCTURE AFTER PGE_2-INDUCED RIPENING IN THE RAT SUGGESTS A PRIMARY ROLE FOR PROTEOGLYCANASES

Helen Feltovich, MD, MS[1]*, Jessie Janowski, BS[1], Nicole DeLance, BS[2], and Edward Chien, MD[3].
[1]Obstetrics and Gynecology, University of Vermont; [2]Pathology, University of Vermont and [3]Obstetrics and Gynecology, Brown University.

Objective: PGE_2 is used clinically and in animal models for cervical ripening, an incompletely understood process. Prior investigation has suggested that disorganization of collagen is critical, however, the mechanisms by which this occurs are elusive. Collagen fibrils are crosslinked in the cervix via proteoglycans, which have been associated with cervical strength. Proteoglycanases disrupt these linkages. Collagenases cleave collagen along its length, which also disturbs collagen structure. Studies evaluating collagenase activity fail to take into account proteoglycanase-mediated disruption of collagen-proteoglycan interactions. It is therefore widely believed that activity of collagenases, and not proteoglycanases, is primarily involved in term cervical ripening, however, this has yet to be clarified. We used transmission electron microscopy to explore cervical ripening-associated microstructural changes within the extracellular matrix in the term pregnant rat in order to better understand the roles of collagenases and proteoglycanases.

Study Design: Timed pregnant Sprague-Dawley rats were treated with intravaginal PGE_2 (n=4) or vehicle (n=4). Indomethacin was administered to prevent endogenous prostaglandin production. PGE_2 was administered in late gestation (day 20). Cervical tissue was harvested 24h post-treatment. Four quadrants from each cervix were prepared for transmission electron microscopy. Four longitudinal images at 12,000x and 4 cross-sectional images at 40,000x were taken from each quadrant. Average measurements of fibril length, diameter and interfibrillary distance were obtained using Metamorph image analysis software. Two-tailed t-tests were used to analyze the data.

Results: Animals treated with PGE_2 demonstrated significantly greater distances between collagen fibrils (10 nm) as compared to controls both at the center of the fiber (30 v. 20nm, $p < 0.0001$) and at the periphery (34 v. 24nm, $p < 0.002$). There were no significant differences in fibril diameter or fibril length in treatment v. control animals.

Conclusion: The increased distance between fibrils in treatment animals suggests remodeling or disruption of proteoglycan links. No change in collagen fibril length was detected, suggesting that fragmentation of collagen plays a less important role in the decreased cervical tensile strength observed in rats after treatment with PGE_2. These data suggest a more critical role for proteoglycanases than for collagenases in the cervical ripening process at term.

ETROP Criteria (EC) versus STOPROP Criteria (SC) for Management of Moderate To Severe Retinopathy of Prematurity (ROP)

JI Hagadorn, AM Furey and TH Nghiem. Dept of Pediatrics, Floating Hospital for Children/Tufts-New England Med Ctr, Boston, MA, United States, 02111.

Background: Criteria have been proposed for treatment of ROP based on the ETROP trial and RM-ROP2 risk model (Arch Ophth 121:1684, Arch Ophth 121:1697, 2003). **Objective:** To estimate the impact of use of EC and RM-ROP2 risk estimates (RE) compared with use of SC for management of eyes with prethreshold or worse ROP (≥Pre). **Design/Methods:** Eye examinations for all infants with ≥Pre at 4 level III NICUs between 1995-2001 were reviewed retrospectively. Eyes were treated using SC for prethreshold (Pre) and threshold (Thr) ROP. Each eye exam was characterized as less than Pre (<Pre), Pre or Thr by SC and as less than type 2 (T2), T2 or type 1 (T1) by EC. RE were calculated and outcomes assessed for all eyes. Favorable outcomes were mature retinal development, vessels reaching Zone 3, or regression to <Pre on two consecutive exams. Unfavorable outcomes were Thr or laser therapy (LT). **Results:** 437 eyes from 241 infants developed ≥Pre. Pre eyes were examined every 5.7±3.2 days. 58 eyes were lost to followup (12 PreT1, 46 PreT2). 7 PreT1 eyes treated early for the ETROP trial were excluded. Of the remaining 372 eyes, treatment of 191 would not have changed had EC been used instead of SC: 84 reached Thr and received LT without an exam meeting PreT1 criteria, 14 received LT at ophthalmologist discretion without meeting Thr criteria, and 94 with PreT2 regressed without reaching T1 or Thr. Using EC, treatment would have changed for 181 eyes having at least one PreT1 exam: For 101 eyes (56% of PreT1) progressing to Thr, LT would have occurred earlier by a median of 7 days (IQR 5, 14 days). 80 (44%) PreT1 eyes that had a favorable outcome would have received unnecessary LT. Use of RE at 1st diagnosis Pre with cut point 0.15 reduced unneeded LT by 45%, while retaining earlier LT for 74% of eyes developing Thr. **Conclusions:** Use of EC would have changed treatment of nearly half of eyes with ≥Pre in this cohort. While most eyes developing Thr could have been targeted for earlier LT using EC, many were not identified as high risk prior to Thr by the described exam schedule. A large minority of PreT1 eyes would have received unnecessary LT. These results support recommendations for twice weekly examination of PreT2. Supplementation of EC with RE may reduce unnecessary LT but would also reduce the number of eyes benefiting from early treatment. Funded by NEI K23 EY/HD00420.

Cyclooxygenase-2 Protein Undergoes Proteolysis Following Activation by Arachidonic Acid and Endocannabinoids. Hewett, J.A, Vidwans, A.S.*, and Hewett, S.J. Department of Neuroscience and *Pediatrics, University of Connecticut Health Center, Farmington, CT 06030 (USA).

Introduction: Cyclooxygenase-2 (COX-2) is a ~70 kD protein residing in endoplasmic reticulum that catalyzes the initial step in the metabolism of arachidonic acid (AA) to bioactive lipid mediators, including prostaglandins. In COS-7 cells over-expressing murine COX-2, a 33-35 kD COX-2 fragment doublet was observed after exposure to AA.

Aim: To characterize COX-2 proteolysis in COS-7 cells overexpressing COX-2.

Methods: COS-7 cells growing in culture were transiently transfected with murine COX-2 cDNA. Cells were then pre-treated with cycloheximide (100 μM) for 1 hour followed by treatment with arachidonic acid (60 μM) with or without other drugs as mentioned. COX-2 activity was measured indirectly by measuring concentration of PGE_2 in the culture supernatants and COX-2 expression was assessed by immunoblot analysis of cell culture lysate.

Results: COX-2 proteolysis occurred rapidly, as early as 1 min after substrate administration, and correlated temporally with catalytic activity. Pretreatment of cells with NS-398, a selective inhibitor of COX-2 activity, blocked AA-induced cleavage. Results with NS-398 were verified using an inactive COX-2 mutant protein, confirming the notion that proteolysis is dependent on enzyme activity. 2-arachidonoyl glycerol, an endocannabinoid substrate for COX-2, also induced COX-2 proteolysis. Activity-dependent COX-2 proteolysis was not unique to transfected COS-7 cells, since it was observed in primary murine astrocytes expressing endogenous COX-2. Finally, neither the proteosome inhibitor, MG-132, nor the golgi toxin, brefeldin A, blocked COX-2 proteolysis.

Conclusions: These results suggest that activity-dependent COX-2 proteolysis occurs locally within the endoplasmic reticulum, perhaps by an endogenous protease, raising the intriguing possibility that local proteolysis may modulate the level of COX-2 in cells [Supported by grants from the NINDS (NS36812) and The Patrick and Catherine Weldon Medical Research Foundation].

Cervical Elastography During Pregnancy: A Feasibility Study

Michael House, M.D., Anastassia Paskaleva, M.S., Ray Chan, Ph.D., and Simona Socrate, Ph.D.

From Tufts-New England Medical Center (M.H.); and Massachusetts Institute of Technology (A.P., R.C., S.S).

Objective: Ultrasound elastography refers to a new technique that aims to use sonographic information to determine mechanical properties of soft tissue in vivo. Currently, an objective method of measuring cervical softness during pregnancy does not exist. Our aim was to determine the feasibility of using elastographic techniques during pregnancy.

Methods: The basic principle is to apply a small amount of pressure to the anterior lip of the cervix using the end of a vaginal probe ultrasound. The end of the probe is covered with a soft, sonographic gel probe cover (GPC) that is held in place with a rubber sleeve. Both the GPC and the rubber sleeve are covered with a latex probe cover, in the standard fashion. As the GPC pushes against the cervix, both the cervix and GPC deform. The mechanical properties of the GPC are known because it is calibrated. Using image-processing techniques, the mechanical properties of the cervix can be derived by comparing the deformation of the GPC to the deformation of the cervix.

Results: A prototype GPC has been developed and tested during pregnancy. The GPC was well tolerated in the clinical environment. Images of the cervix were compared before and after the GPC was attached to the end of the vaginal probe. The GPC did not degrade image quality. Further, deformation of both the GPC and the cervix could be observed and quantified.

Conclusion: Imaging cervical deformation distal to a gel probe cover during pregnancy is feasible. Whether cervical elastography will be a clinically useful technique awaits larger studies.

The recurrence rate of severe perineal tears in vaginal deliveries

Cresta W. Jones, M.D.*, and Eleanor Capeless, M.D., Fletcher Allen Health Care/University of Vermont

OBJECTIVE: Women with severe perineal lacerations may be reluctant to attempt vaginal delivery in a subsequent pregnancy. Our aim was to determine the recurrence risk of severe lacerations and to identify risk factors associated with recurrence.

METHODS: A computerized database was used to identify all nulliparous women with a singleton vertex presentation at greater than 36 weeks gestation with a neonatal weight greater than 2500 grams. Those who had an initial severe laceration were identified (cases) and subsequent pregnancies were reviewed. A cohort of patients was matched to the cases by neonatal weight at initial delivery. Obstetric variables included were pitocin use, labor dystocia, operative delivery and episiotomy. Data analysis was performed to identity risk factors associated with initial and recurrent lacerations. Data was analyzed using Fisher's exact test and t-test using the SAS version 8 statistical analysis package.

RESULTS: 269 cases were compared to 167 controls. The recurrence rate of severe perineal laceration in the cases was 4...1% (95% CI: 2.1-7.2%), and was not statistically different from the rate in the control group, 2.5% (95% CI: 0.7-6.0%). There were no significant associated risk factors for a recurrent severe perineal laceration, although the number of recurrences was small (n=11).

CONCLUSION: Severe perineal laceration in an initial pregnancy is not associated with an increased risk of recurrence in a subsequent pregnancy. Women should be encouraged to attempt vaginal delivery in a subsequent pregnancy.

Resveratrol Mediated Molecular Signaling in Cardioprotection

Prakash Kabbur*[†], Shoji Fukuda[‡], Shigeaki Kaga[‡], Lijun Zhan[‡], Nilanjana Maulik[‡]

Department of Pediatrics[†], Division of Neonatology; Department of Surgery[‡],

Molecular Cardiology Laboratory,

University of Connecticut Medical Center, Farmington, CT, 06030-1110, USA

Abstract: In our study resveratrol (polyphenol) has been identified as being a very important stimulus/agent for the induction of new vessel growth. Occlusion of a main coronary artery depletes the blood supply to the myocardium and subsequently reduces cardiac function, which ultimately leads to heart failure. Progressive, chronic coronary artery occlusion has been shown to induce development of collateral arteries to re-establish and maintain blood flow to the myocardium at risk via the growth of new capillary vessels or angiogenesis. Studies from our laboratory as well as from others have already confirmed the protective role of collaterals against myocardial ischemia and cell death. We have successfully demonstrated in rat myocardial infarction (MI) model effect of resveratrol on significant upregulation of the protein expression profiles of vascular endothelial growth factor (VEGF) and its tyrosine kinase receptor Flk-1, 3 weeks after MI. Resveratrol pretreatment also increased nitric oxide synthase (iNOS and eNOS) along with increased anti-apoptotic and pro-angiogenic factors, NF-κB and SP-1. We were also able to demonstrate increased capillary/arteriolar density as well as improved LV function by resveratrol preconditioning 3 weeks after MI.

FETAL GENDER IN PRETERM DELIVERIES COMPLICATED BY PREECLAMPSIA

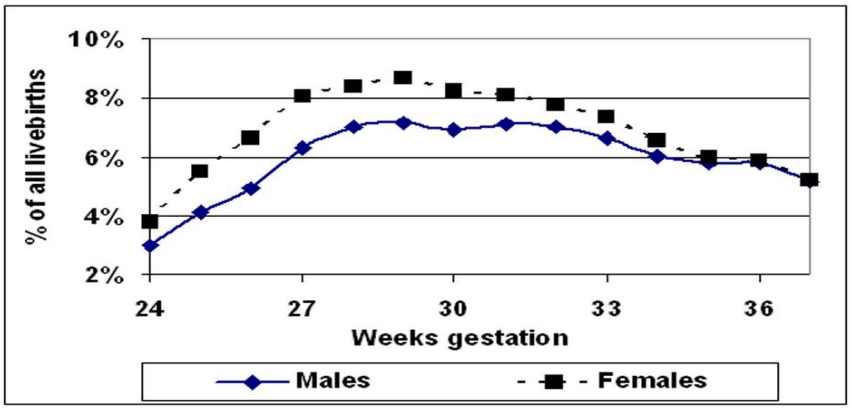
L. KAMINSKY[1]*, J. EGAN[1] AND H. ROQUE[1]

[1]*University of Connecticut, Obstetrics and Gynecology, Farmington, Connecticut*

OBJECTIVE Several studies have reported conflicting information on fetal gender distribution in preterm births to women with preeclampsia. We investigated gender specific preterm delivery rates in pregnancies complicated by preeclampsia in the US.

STUDY DESIGN Using National Center for Health Statistics data, we recorded the number of singleton livebirths (LB) from 1990 to 2002 for women with preeclampsia. Data was stratified by fetal gender and gestational age. Male-to–female sex ratios and gender specific LB percentages of total LB at each week of gestation were determined.

RESULTS There were 305,982 singleton pregnancies with preeclampsia from 24-37 weeks: 159,203 males and 146,779 females. Females were more commonly delivered at each week of gestation from 24-31 weeks, after which males predominated. This fetal sex trend in preeclamptic pregnancies is the opposite of that seen in the general population. When expressed as gender specific and gestational age matched percentages of total LB, females were more likely to be born from 24 to 37 weeks of gestation in pregnancies complicated by preeclampsia (see Figure 1).



Gender specific percentages of total LB by gestational age in preeclamptic pregnancies.

CONCLUSION Our study demonstrated that preterm preeclamptic pregnancies were associated with a higher incidence of female LB compared with the male predominance in the general population. The mechanism of this gender effect is unclear but may provide insight into pathophysiology of prematurity in preeclampsia.

Ventilator-Associated Pneumonia in the NICU- A Study of Rates, Risk Factors and Outcomes.

Folasade Kehinde*, Naveed Hussain, Ted Rosenkrantz. University of Connecticut Health Center, Farmington, CT.

Background: Ventilator-Associated Pneumonia (VAP) is recognized as a major risk factor for morbidity and mortality in the adult ICU population, but very few studies exist in the older pediatric population and especially in the newborn. **Objectives:** To describe the incidence, risk factors and outcomes associated with VAP in a newborn population. **Methods:** A retrospective study was conducted at the Neonatal Intensive Care Unit (NICU) of the John Dempsey Hospital. All infants admitted to the NICU between July 1, 2002 and June 30, 2004 who required assisted ventilation for > 48 hr were identified using a patient database. The medical records of all infants with a presumptive or confirmed diagnosis of pneumonia were reviewed for: demographic data, total ventilator days, duration of ventilation before development of pneumonia, presence of signs and symptoms of VAP as defined by the National Nosocomial Infections Surveillance system (NNIS), diagnosis of RDS and surfactant administration, patent ductus arteriosus (PDA), colonization with *Ureaplasma or Mycoplasma*, and duration of indwelling central lines. Outcomes measured were bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), periventricular leukomalacia (PVL), necrotizing enterocolitis (NEC), length of stay (LOS) and death. Infants with genetic syndromes, congenital abnormalities and congenital pneumonia were excluded. Univariate and multivariate analyses were used to determine significance of risk factors and effects on outcomes. **Results:** 913 infants were admitted. 357 (39%) required intubation for > 48 hrs. 94 infants had a presumptive or confirmed diagnosis of pneumonia. Only 38 (10.6%) met NNIS criteria for VAP. Mean GA for patients with VAP was 26 ± 3wk. There was no gender difference. VAP was due to gram (-) organisms (47%) and gram (+) organisms (52%). Univariate analysis revealed the following risk factors: {BW <1500g ($p< 0.0001$), RDS ($p < 0.0001$), PDA (p 0.0002), endotracheal intubation >8d ($p <0.0001$), duration of indwelling central lines (40d vs 15d $p <0.0001$)} and outcomes {LOS (97d vs 37d $p<0.0001$), mortality (17% vs 0% $p < 0.0019$)}. *Ureaplasma* or *Mycoplasma* colonization, NEC and PVL were not associated with VAP. With multivariate logistic regression analysis, only GA and BW remained independent predictors for VAP and mortality. **Conclusions:** In the NICU, VAP occurs in 10.6% of infants requiring assisted ventilation for > 48 hrs. Incidence is highest after eight days of assisted ventilation. GA and VLBW are independent predictors of VAP. Significant morbidity and mortality are associated with VAP. **Significance:** This study demonstrates significant morbidity and mortality in VLBW infants who remained on the ventilator for >8d. This underscores the need for increased efforts towards earlier extubation in this high-risk population.

Etiology And Clinical Study Of Pneumonia In Newborn - Analysis Of 169 Cases

Li Jian, Song Yuan-yuan, Li Xiao-jing, et al. Maternal and Infant Hospital of Guangzhou, Guangdong Province, Peoples Republic of China

Objective To study the pathogens and clinical manifestations of pneumonia in newborns. **Methods** 169 neonates were diagnosed with pneumonia. The symptoms of pneumonia in the newborn included shortness of breath, cyanosis, and cough. In these infants, serum viral antibodies, including respiratory syncytial virus antibody, adenovius antibody, coxsackie virus antibody, cytomegalovirus antibody, and nasopharyngeal virus antibody were measured in respiratory tract secretions by ELISA. Mycoplasma pneumoniae antibody was measured in throat swabs by granular agglutinating test, chlamydia trachomatis (CT) antigen by was measured by the gold standard method. Ureaplasma urealyticum was examined in throat swabs and sputum by culture. Ten pathogens including above described virus, influenza virus, and parainfluenza virus were cultured in several patients in which antibody and antigen were measured. **Results** Only virus antibody IgM or IgA were measured in most cases. The positive rate was 6.25%. The positive rate of Mycoplasma IgM was 4.71%, Ureaplasma urealyticum culture 41.67%, CT (antigen) 16.67%, but CT (sputum culture) 44.9%. Ten pathogens of pneumonia were studied in 25 patients. Viral antigen was positive in 23 cases in 15 newborns, viral antibody was positive in 4 cases in 2 newborns. Sputum culture in lower respiratory tract positive rate was 92%. **Conclusion** This study of pneumonia in newborns suggests low viral antibody positive rate. When considering viral pneumonia, antigen should be measured if possible. Mycoplasma infection was mostly Ureaplasma urealyticum infection. Chlamydia infection was also not unusual. This should be emphasized. Sputum culture was still an important examination method.

ErbB Receptor Signaling in Fetal Lung Development: Do Mesenchyme and Epithelium Treat Each Other Differently?

Washa Liu, MD [1*], Sandy L Murray, PhD [1], Christiane EL Dammann, MD [1,2] and Heber C Nielsen, MD [1]

[1] Pediatrics, Floating Hospital at Tufts-NEMC, Boston, MA 02111, United States and [2] Pediatrics, Hannover Medical School, Hannover 30625, Germany.

Background: Mesenchyme-epithelial communication is important in fetal lung cell proliferation and maturation. Maturation activity, described as an unidentified factor in fibroblast conditioned media (FCM), stimulates alveolar type II (T2) cell surfactant synthesis. We have shown that the ErbB receptor ligands epidermal growth factor (EGF), transforming growth factor (TGF), and neuregulin 1 (NRG) influence mesenchyme-T2 cell communication. For example, FCM activates ErbB receptors in stimulating T2 cell surfactant synthesis. NRG mimics FCM activity, and both are blocked by NRG and ErbB receptor antibodies. Mesenchymal control of T2 cell proliferation is also necessary for fetal lung growth and maturation. The mechanisms of mesenchyme-T2 cell communication in fetal lung maturation are unclear.

Objective: We hypothesized that conditioned media from fetal lung fibroblasts (FCM) and T2 cells (T2CM) differentially regulate ErbB receptor signaling in fetal T2 cells and fibroblasts, respectively.

Design/Methods: Day 19 fetal rat lung fibroblasts and T2 cells were cultured to confluence, then serum starved for 24 hrs. Fibroblasts were treated with T2CM and T2 cells with FCM for another 24 hours. For positive controls, cells were stimulated with EGF (100ng/ml), TGF (100ng/ml), or NRG1 (33 nmol) for 5 min. Western blots of cell lysates were probed with antiphosphotyrosine and ErbB receptor antibodies.

Results: Compared to no treatment, FCM increased phosphorylation of ErbB4, ErbB2 and ErbB1 by 30% in T2 cells, similar to the growth factors. T2CM stimulated fibroblast ErbB4 and ErbB2 phosphorylation 2-fold, but inhibited ErbB1 phosphorylation.

Conclusions: Fetal lung fibroblasts and T2 cells show clear differences in stimulation by one cell type of ErbB receptor phosphorylation in the other cell type. Mesenchymal-epithelial communication in late fetal lung development controlling cell proliferation and differentiation may act via paracrine effects mediated via ErbB receptors. We speculate that the observed differences in ErbB receptor phosphorylation reflect differential control of proliferation and differentiation. Funding: NIH HL37930, NIH HL 04436, Hood Foundation, Peabody Foundation.

Antibody Inhibition of ErbB Receptors in Pulmonary Alveolar Type II Cell Lines

Sandra L Murray, PhD [1], Washa Liu*, MD [1], Lucia D Pham, BS [1], Sujatha Ramadurai, MD [1], MaryAnn V Volpe, MD [1], Christiane E.L. Dammann, MD [2,1] and Heber C Nielsen, MD [1]. [1] Pediatrics, Tufts-New England Medical Center, Boston, MA, United States, 02111 and [2] Pediatrics, Hannover Medical School, Hannover, Germany.

Background: Fetal lung maturation involves fibroblast-type II epithelial cell communication through erbB receptors (erbB1, erbB2, erbB3, erbB4). ErbB ligands (epidermal growth factor (EGF); neuregulin (NRG)) stimulate this process. Ligand binding induces ErbB homo- and heterodimer formation and phosphorylation, activating multiple signal transduction cascades. Specific dimers involved in fetal lung surfactant synthesis are unknown. We showed that erbB receptor blocking antibodies (Abs) reduced ligand-induced DSPC synthesis (a measure of surfactant synthesis) in the human lung epithelial carcinoma line, A549. Anti-erbB Abs alone or in combination differentially decreased synthesis induced by EGF, NRG or fibroblast conditioned media (FCM).

Objective: We hypothesize that erbB receptor dimer formation necessary for DSPC synthesis differs between tumor-derived vs non-transformed lung cell lines.

Design/Methods: MLE12 cells (derived from SV40 transgenic mouse pulmonary tumors) and L2 cells (non-transformed cells derived from normal adult rat lung) were preincubated with inhibitory antibodies directed against extracellular epitopes of the erbB receptors, individually or in combination. Cells were then treated overnight with EGF (10ng/ml), NRG(10nM) or FCM, and ^{3}H-choline to measure DSPC synthesis.

Results: Anti-erbB3 decreased DSPC synthesis in all ligand-treated MLE12 cells by 25-35%. Anti-erbB1+anti-erbB4 affected only FCM-treated cells (40% decrease); anti-erbB2+anti-erbB4 reduced DSPC in all ligand-treated cells by 65%. NRG and FCM stimulated DSPC synthesis in L2 cells by 50%. Preincubation with anti-erbB3 or 4 further stimulated incorporation by 200% (NRG) and 300% (FCM).

Conclusions: A549, MLE12 and L2 cells have all been used as pulmonary type II cell models. Inhibitory erbB Abs differentially affect surfactant synthesis in these different cell lines. Inhibitory Abs against NRG-binding receptors B3 and B4 are particularly effective, alone or in combination with anti-erbB2, in tumor-derived lines. In contrast, the same Abs were stimulatory in non-transformed L2 cells from normal adult lung, particularly in the presence of FCM. We speculate that effects on surfactant synthesis are signaled through different ErbB dimers depending on the cell line used. NIH HL37930, HL04436, Hood and Peabody Foundations.

Nursing Opinions and Practices of Oxygenation in Prematures: The NOPOP Study

T H Nghiem* [1], J I Hagadorn [1], S Syke [1], B MacKinnon [2] and C H Cole [2]. [1] Newborn Medicine, Tufts - New England Medical Center and [2] Neonatology, Beth Israel-Deaconess Medical Center

Background: Oxygen is a common but controversial therapy for infants <28 weeks (extremely low gestational age neonates, ELGANs). Optimal oxygen saturation for these infants is unknown. In this uncertain climate, personal opinions and preferences may influence clinical practice. Data are limited regarding the relationship between nursing opinions and oxygen management practices in ELGANs. **Objective:** To document neonatal nursing opinions and stated practices regarding pulse oximetry (SpO2) ranges for ELGANs during the first 4 weeks of life, and compare to neonatal intensive care unit (NICU) policies. **Design/Methods:** This was a web-based survey of nurses from all US Level III NICUs with neonatal-perinatal fellowship programs. For each center, information was collected regarding NICU and nursing characteristics, SpO2 policy range, and nursing practice and opinion regarding SpO2 range. Mean ± SD values for SpO2 in practice, opinion, and policy were compared using the independent or paired samples t-test as appropriate. **Results:** Fifty-nine NICUs (60% of eligible centers) submitted 2823 surveys (45% of eligible nurses). In all, 68% of participating centers had a written policy or guideline for ELGAN SpO2 range.

NICU SpO2 Policy	Yes (NICUs=40)		No (NICUs=19)		p-value
	Lower Limit	Upper Limit	Lower Limit	Upper Limit	
SpO2 Policy (Mean SpO2 ± SD)	86 ± 3	94 ± 2 ∃⊥	NA	NA	
RN Stated Practice (Mean SpO2 ± SD)	87 ± 2 *^	95 ± 1 **∃	89 ± 1 *^	96 ± 2 **^	* <.001 ** .009
RN Opinion (Mean SpO2 ± SD)	86 ± 2 *^	95 ± 1 ⊥	88 ± 1 *^	96 ± 1 ^	* <.001
p-value	^ <.001	∃ .001 ⊥ <.001	^ <.001	^ .001	

Conclusions: Moderate but significant differences exist between neonatal nursing opinions, practices, and unit policies in US NICUs regarding SpO2 limits for ELGANs. Nurses believe that the lower limit of SpO2 should be lower than they currently practice. At centers with a policy, nursing practice includes higher SpO2 values than specified by their policy upper limit. Nurses at these centers believe that acceptable saturation range should include higher upper limits than specified by current policy. These opinions, preferences, and practices have the potential to affect compliance with unit policies or study protocols specifying a target SpO2 range. Funded by GCRC/Natl Center for Research Resources MO1-RR00054

Is a Third Trimester Growth Scan Necessary for Fetuses with an Isolated SUA?

Panzi, Shannon*; O'Brien, Barbara* House, Michael; Chelmow, David; Craigo, Sabrina

Tufts- New England Medical Center

OBJECTIVE: To determine if a third trimester ultrasound in fetuses with an isolated single umbilical artery (SUA) is beneficial.

STUDY DESIGN: A retrospective cohort study was conducted on all patients with an isolated SUA who were identified from an ultrasound database between 1999-2003. A third trimester sonogram for growth was standard for these patients. Exclusion criteria included multiple gestations, maternal hypertension or diabetes, fetuses with oligohydramnios, polyhydramnios, structural anomalies, chromosomal abnormalities, and abnormal serum screening. Intrauterine growth restriction (IUGR) was defined as an EFW < 10 percentile using Hadlock tables. Fetuses with minimal hydronephrosis were selected as the control group because they routinely had a third trimester scan and were not at risk for IUGR. Multivariate models were constructed to control for confounders, such as age, gravidity, parity, EFW, and weight percentile.

RESULTS: Of 253 patients with SUA, 99 (39%) met the inclusion criteria. Of 636 patients with minimal hydronephrosis 200 (31%) met the inclusion criteria. The incidence of IUGR among fetuses with SUA was 5% (5/99), compared to 2% (4/200) among fetuses with mild hydronephrosis (RR=2.5, 95% CI 0.69 - 9.2, p=0.16). Multivariable linear regression revealed that SUA was associated with a decrease in EFW of 107 gm (95% CI 73 -141 gm, p= 0.002). Logistic regression revealed a nonsignificant trend toward an increase in IUGR among SUA fetuses, (adjusted odds ratio=2.9, 95% CI 0.72 - 12, p=0.13). All cases of IUGR with SUA (5) occurred after 34 weeks.

CONCLUSION: There is a statistically significant decrease in fetal weight and a trend toward an increased incidence of IUGR in fetuses with isolated SUA. If a growth scan is recommended in fetuses with isolated SUA, our data suggests that it should be performed after 34 weeks.

DO SYSTEMIC ANTIFUNGAL MEDICATIONS CAUSE HEPATOTOXICITY IN NEONATES WITH FUNGAL SEPSIS? A RETROSPECTIVE REVIEW.

Authors: Shama Praveen*, Peter Krause, Kathleen Mckay, Isabella Knox. Institutions: Divisions of Neonatology, Infectious Disease and Biostatistics, University of Connecticut Health Center (UCHC), Farmington, CT and Connecticut Childrens' Medical Center (CCMC), Hartford,CT.

Background: Hepatotoxicity related to amphotericin B therapy is rare with only three case reports found in the literature in adults with none described in the neonatal population. **Objectives:** To determine if treatment of fungal sepsis in neonates with amphotericin B is associated with laboratory evidence of hepatocellular injury (HCI). **Methods:** All neonates with fungal sepsis admitted to the neonatal intensive care units (NICU) at UCHC and CCMC from January 1998 through December 2003 were identified using a neonatal database and medical records were reviewed. Demographic variables and liver function tests obtained within 2 weeks prior to and 2 weeks after the diagnosis of fungal sepsis were recorded. HCI was defined as an abnormality of direct bilirubin (DB) and/ or either aspartate aminotransferase (AST) or alanine aminotransferase (ALT). **Results:** Fifty four infants with fungal sepsis were identified; 37 charts were reviewed. All records contained at least one laboratory parameter of interest**.** All of the study patients received systemic amphotericin B and one received caspofungin in addition**.** Seven of the 37 (23%) patients had evidence of HCI and 30 patients had no evidence of HCI. Demographic variables were compared between the two groups (Table-1). Mean birth weight of the infants with HCI was greater than in infants without HCI. The mean % increase in liver functions from pre-treatment values in patients with HCI was DB 66%, AST 63% and ALT 74%. Among the 7 patients with HCI, the greatest elevation in laboratory parameters was seen in the patient who received dual therapy.

Table 1	**HCI (n=7)**	**No HCI (n=30)**	**P value**
GA(wk) mean ± SD	27.7 ± 12.5	26.2 ± 2.3	0.18
BW(gms) mean ± SD	1165.7± 589.3	865.2 ± 225.0	0.03
Race(W) n(%)	14 (%)	47 %	0.2
Gender(M) n(%)	57 (%)	66 %	0.63
Age at onset (days) mean ± SD	29.2 ± 13.4	25.5 ± 14.7	0.54

Conclusion: HCI is common in neonates with fungal sepsis treated with amphotericin B. Because liver injury is multifactorial, it is not clear if this is due to amphotericin B toxicity alone. The finding of a higher birth weight in infants with HCI is intriguing and requires further study. The fact that one infant treated with 2 drugs had more evidence of liver injury suggests a possible role of caspofungin in its causation. Before caspofungin is used more widely in neonates, assessment of its potential for hepatotoxicity must be made.

Alveolar Epithelial Cells Synthesize Retinoids from β-Carotene: A New Regulatory Pathway in Lung Development

Lewis P. Rubin, Amy C. Tsai, Brandi A. Deggins, Paula V. Freedman, Monique E. De Paepe, Xiaoming Gong. Departments of Pediatrics and Pathology & Laboratory Medicine, Program in Fetal Medicine, Women & Infants Hospital/Brown Medical School, Providence, RI.

Background: Vitamin A is essential in lung morphogenesis and pulmonary alveolar differentiation. The key step in vitamin A formation is oxidative cleavage of β-carotene (βC) by the enzyme β,β-carotene-15,15-oxygenase (βCO1). βC-9,10-oxygenase (βCO2) catalyzes an excentric cleavage pathway. Intestine and liver are the principal sites for βC→vitamin A (retinoid) metabolism. Little is known about extra-enteric βCO1/2 expression or function.

Objective: We tested the hypotheses that βCO1 and βCO2 (*1*) have distinct patterns of pulmonary cell-specific and developmental expression and (*2*) regulate retinoic acid (RA) synthesis in the lung.

Design/Methods: βCO1 and βCO2 mRNA levels were assayed by northern blot and qRT-PCR. ~1kb of human βCO1 proximal promoter sequence was cloned into pGL3 and transfected into A549 cells. Peptide-specific βCO1 and βCO2 rabbit anti-human polyclonal antibodies were raised to unique enzyme peptide sequences, affinity purified and validated for Western blot (WB) and immunohistochemistry (IHC). βC→retinoid (RA) metabolism was measured by HPLC or by a cotransfected pRARE-luc reporter activity.

Results: We detected βCO1 and βCO2 mRNA and protein in human and mouse lung. In the mouse, βCO1 (but not βCO2) expression is developmentally regulated (E17-P5). βCO1 and βCO2 are expressed predominantly in type II alveolar epithelial cells (AECs). Human AEC-like A549 cells also express these enzymes and metabolize βC to biologically active RA isomers. Dexamethasone treatment (Dex, 0.1μM x 24h) to promote A549 cell differentiation markedly inhibited βCO1 mRNA and protein accumulation and βCO1-luc promoter activity but ↑ed βCO2 expression ≥4-fold.

Conclusions: AECs express βCO1 and βCO2. Pulmonary βCO1 and βCO2 gene expression are differently regulated during development and by extracellular signals including Dex. This discovery that

The role of prenatal magnetic resonance imaging in fetuses with sonographically identified intracranial anomalies. Danielle Salhany, B.S.*, Joseph R. Wax, M.D., Angelina Cartin, Michael G. Pinette, M.D., Jacquelyn Blackstone, D.O. Division of Maternal-Fetal Medicine, Department of Ob/Gyn, Maine Medical Center, Portland, Maine.

Objective: To assess the impact of prenatal magnetic resonance imaging (MRI) on the diagnosis and management of fetuses with sonographically identified intracranial anomalies.

Methods: We performed an institutionally exempted retrospective study of all women undergoing prenatal MRI from 1/1/2002-10/15/2004 for further evaluation of sonographically detected intracranial anomalies. All ultrasound examinations were performed by registered sonographers, under Maternal-Fetal Medicine specialist supervision using HDI 3000 or HDI 5000 (ATL, Bothell, WA), or Voluson 730 (GE Medical Systems, Milwaukee, WI) equipment. MRI was performed using single-shot fast spin-echo imaging to obtain coronal, axial, and sagittal imaging through the fetal brain. We then assessed whether the MRI confirmed the ultrasound diagnosis, amended the ultrasound diagnosis, or altered pregnancy management.

Results: A total of 12 patients, aged 22-41 years, underwent ultrasound examination at 17.6-34.0 weeks' gestation. Referral indications were suspected intracranial anomalies (n=6), other suspected anomalies (n=2), maternal age $\geq$ 35, adverse reproductive history, abnormal second trimester maternal serum screen, and multiple gestation. MRI performed from 21.7-37.0 weeks' gestation confirmed the sonographic diagnosis in 2 (16.7%) cases, amended the ultrasound diagnosis in 10 (83.3%) cases, and led to a care plan change in 1 (8.3%) pregnancy.

Conclusion: Prenatal MRI is a valuable adjunct to ultrasound in evaluating and managing fetuses with intracranial anomalies.

The Influence of Gestational Diabetes Mellitus on Perineonates

Song Yanyan, Zhou Hongmei, and Wu Yuqun, et.al. The Infant and Maternal Hospital of Guangzhou, Neonatal Department, Perineonatal Institute Laboratory, Guangdong Province, Peoples Republic of China.

Objective: To study the influence of gestational diabetes mellitus (GDM) on perineonates. Methods: Blood was taken from 32 neonates of GDM and from 20 healthy neonates as controls, in order to test blood insulin, cortisol and insulin-like growth factor-1 (IGF-1).

Results: 1) The early insulin, cortisol and IGF-1 of GDM neonates were significantly higher than that of the control neonates ($P<0.05$); 2) The early blood sugar of GDM mothers' neonates was not significantly different compared to the control group. 3) The presence of a Cushing-like appearance, neonatal RDS, and macrosomia were still high in the GDM infants.

Conclusions: Changes in the GDM mothers' endocrine system can influence the endocrine system of their fetus, as shown by neonatal appearance and the incidence of RDS. Metabolically this is evident as elevated blood insulin, cortisol and IGF-1 levels in the neonate. These metabolic changes will influence neonates' growth, increase their weight and may influence their quality of life. Our data indicate that a high level of concern for these neonates is appropriate.

Clinical Study of the Pathological Changes in Placentas of Mothers with Edema-Proteinuria-Hypertension Syndrome and the Effect on the Neonate

Song yanyan, Li Xiaojing, Wu Yuqun et al. The Infant and Maternal Hospital of Guangzhou, Neonatal Department, Perineonatal Institute Laboratory, Guangdong Province, Peoples Republic of China.

Objective: To understand the association of pathological changes of the uterus and placenta of patients with edema-proteinuria–hypertension syndrome (EPH) with injury of perineonates. ***Methods:*** The placentas of mothers with EPH were examined pathologically. Brain ultrasonography-B, ABR and EEG examination were done on neonates**.**

Results: In the EPH group, fibrous necrosis of the villi of uterus and placenta was present in >25% ; increased syncytium cell nodules was seen in >70%. The occurrence of increased terminal villus vessels and congestion, placental and decidual cell inflammation, partial infarction of the placenta, thicker spiral arterial vessels of uterus with narrow lumens, peripheral congestion, and hemorrhage umbilical artery were all significantly higher than in a control group, $P<0.05$. The villous space stenosis was greater in mothers with moderate and severe EPH compared to mothers with minimal EPH ($P<0.05$). Abnormalities of ultrasonography-B of brain, EEG, and ABR were more often seen in infants of the EPH group compared to controls ($P<0.05$). The occurrence of premature birth and low birth weight was significantly higher in the EPH group compared to the control group ($P<0.05$).

Conclusion: Chronic pathological changes in the uterus and placenta of EPH pregnancies affect the exchange of blood and nutrition between fetus and mother, resulting in intrauterine chronic hypoxia. This may result in brain damage and changes of function and structure of the central nervous system that influences the prognosis of neonates. This suggests that we should emphasize perinatal health care, and that a careful neonatal nervous system examination such as ultrasonography-B of brain, EEG, and ABR should be performed early. This will help with prompt diagnosis and allow early intervention to help the prognosis for these neonates.

Echocardiography and Advanced Maternal Age: Roman Starikov* MD, Glenn Markenson MD, and Fadi Bsat MD. Baystate Medical Center, Division of Maternal Fetal Medicine, Springfield MA.

Women 35 years or older are offered amniocentesis due to an increased risk of fetal chromosomal anomalies. However, there is a risk of pregnancy loss with this procedure. Up to 50% of fetuses with aneuploidy will have a cardiac structural abnormality. As a result, non-invasive tools such as genetic sonography and fetal echocardiography have been used to screen for fetal chromosomal anomalies. The purpose of this study is to determine if a fetal echocardiogram increases the detection rate of suspected fetal cardiac defects when a genetic sonogram reports normal fetal cardiac anatomy in women at increased risk for fetal aneuploidy due to advanced maternal age.

Research Design and Methods: Institutional Review Board approval was obtained. A retrospective study was performed, utilizing the Baystate Medical Center Perinatal Ultrasound Database. The database was searched for women 35 years of age or older who had a genetic sonogram between 16-20 weeks gestation with no major cardiac abnormality seen, followed by fetal echocardiogram. Major cardiac anomalies consisted of structural cardiac defects. Minor cardiac findings included echogenic intracardiac foci, arrhythmias and pericardial effusions.

Results: Between November 2001 and October 2004, 86 patients met the inclusion criteria. None of the patients that had a normal cardiac evaluation at the time of the genetic sonogram was suspected of having a major structural cardiac defect at the time of the fetal echocardiogram. A total of 8 minor cardiac findings were found at the time of the genetic sonogram: 6 echogenic foci and 2 pericardial effusions. Ten minor cardiac findings were noted during the fetal echocardiogram. One fetus had pericardial effusion which was also seen at the genetic sonogram, and 9 others had an echogenic focus, of which 6 were seen already during the genetic sonogram. The sensitivity, specificity, positive and negative predictive values of a genetic sonogram to predict minor cardiac findings are: 70%, 99%, 86% and 96%. The positive likelihood ratio is 7 and the negative likelihood ratio is 0.3.

Discussion: This study was limited by the small sample size. Nevertheless, it appears that the likelihood of finding a major cardiac anomaly after a normal genetic sonogram in an advanced maternal age population is low. A fetal echocardiogram may detect additional minor cardiac findings such as echogenic foci. Patients with advanced maternal age have a much higher risk of aneuploidy based on their age compared to the finding of an echogenic focus; therefore the added value of this weak marker for a chromosomal anomaly is limited. Studies with larger numbers of subjects need to be performed to determine the clinical value of a fetal echocardiogram after a normal genetic sonogram.

Application of cDNA micro arrays in profiling gene expression in ischemic mouse myocardium.

R. Vidavalur*, S Kaga, L Zhan, W Kruger, N Maulik.

University of Connecticut Health Center, Farmington, CT06030.

Introduction: The tissue response to ischemic cardiac stress is accompanied by changes in gene expression. We have used cDNA microarrays to study gene expression, as this procedure allows the evaluation of transcriptional response for thousands of genes simultaneously. Sildenafil is a selective inhibitor of phosphodiesterase-5 (PDE-5). It increases cellular cGMP levels and helps in initiating protein phosphorylation cascade. It has been demonstrated to have potent cardioprotective effects against ischemia-reperfusion injury in recent animal model experiments from our laboratory.

Objective: 1. to study the genes involved in early hours of ischemia in normal mouse heart and (2) to study the changes in gene expression of myocardium of sildenafil treated mice in vivo after two hours of ischemia.

Methods: Following myocardial ischemia, gene expression in the untreated and treated tissue was studied using our micro array core facility with NIA 15K mouse gene library. For image and data analysis, we used the Gleams micro array program and an in house developed improved cluster analysis algorithm.

Results: By cluster analysis, we identified 66 genes showing differential expression as well as a number of genes that are up regulated and down regulated.

Conclusions: There was 6-9 fold down regulation in genes involving g-protein signaling and apoptotic activity and 1-2 folds up regulation in protein, ATP, DNA binding genes in sildenafil treated group. Our results provide a detailed view of the early evolving transcriptional changes in the ischemic myocardium. These responses may represent the balance between the cardioprotective and degenerative processes that accompany myocardial ischemia.

Hoxb-5 Down Regulation Alters Tenascin-C and FGF10 Expression Patterns in Psseudoglandular Period Fetal Mouse Lung

MaryAnn V. Volpe, M.D., Soujanya Rallabandi, Lucia D. Pham, Sandra L. Murray, Ph.D., Sujatha Ramadurai, M.D., and Heber C. Nielsen, M.D. Pediatrics, Tufts-New England Medical Center, Boston, MA

Background: Vertebrate evolution required simultaneous development of limb and lungs, suggesting related morphogenetic mechanisms in their embryonic patterning. We showed that the Hox gene Hoxb-5 influences airway patterning during lung morphogenesis. Limb patterning is in part determined by interaction of Hox genes and fibroblast growth factors (FGF) affecting cell adhesion and cell fate. The coordination of these pathways in lung development is unknown.

Objective: Determine if Hoxb-5 effects on airway patterning are coordinate with effects on the extracellular matrix (ECM) and spatial restriction of FGF expression. We hypothesized that down regulation of Hoxb-5 leads to decreased tenascin-C and altered expression patterns of FGF10.

Design/Methods: Gestational day 14 (d14) fetal mouse lung fibroblasts and whole lung cultures were transfected with Hoxb-5 specific or control (scrambled and GAPDH) small interfering RNA's (siRNA), or vehicle alone. Optimal Hoxb-5 siRNA sequence and dose was determined. After 72 hrs of treatment, Hoxb-5, tenascin-C and FGF10 protein expression were evaluated using Western blot analysis with densitometry, and immunocytochemistry. Hoxa-5 protein, a close paralogue of Hoxb-5, was also evaluated.

Results: In fibroblast cultures, Hoxb-5 siRNA treatment decreased Hoxb-5 protein expression by 50% compared to controls, without affecting Hoxa-5 protein levels. This inhibition of Hoxb-5 led to a 50% reduction in tenascin-C and 30% reduction in FGF10 protein levels. Compared to controls, Hoxb-5 siRNA-treated d14 whole lung cultures were smaller with shorter developing airways. Immunostaining showed negligible Hoxb-5 protein, minimal tenascin-C and loss of FGF10 spatial restriction. FGF10 was more diffusely expressed in mesenchyme around branching airway tips.

Conclusion: Hoxb-5 expression in fetal lung fibroblasts regulates protein levels of tenascin-C and FGF10, and spatially restricted FGF10 expression. We speculate that Hoxb-5 regulates lung airway development through tenascin-C modulation of ECM around branching airways. ECM changes then affect mesenchymal-epithelial cell communication to alter spatial restriction of FGF10. Supported by NIH HD38419, HD04478, HL37930, and Zucker Women Scholars Award.

Mechanical Stretch Activates Heterotrimeric G-Protein Alpha S Subunit And C-AMP Pathway In Fetal Lung Type II Cells

Yulian Wang, Nicole Lee, Ophira Silbert, Juan Sanchez-Esteban. Department of Pediatrics, Women & Infants' Hospital Rhode Island and Brown Medical School, Providence, RI.

Mechanical forces are critical for fetal lung development. However, the cell surface receptors and signaling pathways that transduce mechanical cues remain unknown. We hypothesized that β_2 adrenergic receptor (β_2AR)-cAMP signaling pathway mediates stretch-induced lung differentiation. Fetal rat pulmonary epithelial cells on E19 (term=E22) were isolated and cultured on silastic membranes precoated with collagen I. E19 monolayers were then subjected to mechanical stretch for varying intervals to simulate fetal breathing movements. Unstretched samples were used as controls. Fetal epithelial cell differentiation was assessed by surfactant protein C (SP-C) mRNA expression. Activation of the G-protein αs subunit was studied by co-IP and western blot. cAMP pathway induction was analyzed by western blot and ELISA. Gene expression of components of this cascade was assessed by real time-PCR. Our results show that mechanical stretch activated G-αs subunit by 2-fold after 1 min of cyclic stretch. Likewise, cAMP and the transcription factor CREB were maximally induced after 5 min of stretch. Interestingly, mechanical stretch did not activate PKA. The addition of cAMP agonists Forskolin or IBMX increased SP-C expression. Pharmacologic inhibition of the β_2AR with propanolol or ICI decreased stretch-induced SP-C mRNA when compared to unstretched, control samples. Finally, adenylyl cyclase and CREB genes were upregulated after 16 h of mechanical stretch. These experiments suggest that β_2AR may function as mechanoreceptor in fetal lung differentiation. Our data also indicate that mechanical forces activate G-αs, cAMP and CREB probably through a PKA-independent signaling pathway, providing further insights as to how mechanical forces influence lung development.

Reducing pain with genetic amniocentesis—A randomized trial of subfreezing versus room temperature needles. Joseph R, Wax, M.D., Michael G. Pinette, M.D., Molly Carpenter, M.S., Renée Chard, M.S., Jacquelyn Blackstone, D.O., Angelina Cartin. Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Maine Medical Center, Portland, Maine

Objective: To determine whether pain associated with second trimester genetic amniocentesis is decreased by using subfreezing rather than room temperature needles. **Methods:** Consenting subjects were randomized to a minus 14°C or room temperature (20-22°C) 22 gauge spinal needle in this institutionally approved study. Patients, blinded to allocation, recorded anticipated and actual pain before and after the procedure, respectively, using a 10 cm visual analog scale with 0 = no pain and 10 = excruciating pain. All procedures were performed similarly under ultrasound guidance by three physicians. 29 subjects in each arm were needed to provide 80% power at the 0.05 level to detect a 1.4 cm difference in actual pain between study groups. **Results:** Thirty-three subjects were randomized to room temperature and 29 subjects to subfreezing needles. Demographics and procedural characteristics were similar in both groups. Anticipated pain was similar in room temperature, 5.1 (95% CI 4.5-5.7) ± 1.7, and subfreezing groups, 4.9 (95% CI 4.1-5.6) ± 2.0, respectively ($p = 0.6$). Actual pain was also similar in the room temperature, 3.6 (95% CI 2.9-4.3) ± 2.0, and subfreezing groups, 2.8 (95% CI 2.0-3.6) ± 2.0, respectively ($p = 0.14$). Similar numbers of subjects in the room temperature and subfreezing groups reported less actual pain (20 vs. 18), greater actual pain (4 vs. 4) or no difference in pain (9 vs. 5) than anticipated ($p = 0.6$). **Conclusion:** A subfreezing 22 gauge spinal needle does not decrease pain associated with second trimester genetic amniocentesis.

Patient Choice Cesarean—The Maine Experience

Joseph R.Wax, M.D., Angelina Cartin, Michael G. Pinette, M.D., Jacquelyn Blackstone, D.O.
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Maine Medical Center, Portland, Maine

Objective: To determine Maine obstetricians' attitudes and practices regarding patient choice cesarean. **Methods:** A questionnaire was sent to all Maine Fellows of the American College of Obstetricians and Gynecologists (ACOG) actively practicing obstetrics, after receiving institutional review exemption. Data were analyzed using descriptive statistics, Fisher exact, or Chi-square tests. **Results:** Seventy-eight of 110 (70.9%) fellows responded. Sixty of 71 (84.5%) respondents performed or were willing to perform choice cesarean. However, 15/71 (21.1%) preferred cesarean for themselves (females) or partners (males). Indications included urinary continence (53.3%), adverse prior birth experience (41.7%), anal continence (35.0%), concern for fetal death/injury (33.3%), and fear of childbirth, preservation of sexual function, or pelvic organ prolapse (26.7% each). Less frequent were pain (11.7%), convenience (8.3%), and provider availability (10.0%). 82.1% believed medical evidence and 85.9% believed ethical issues sometimes or always supported choice cesarean. Responses were similar by gender, age, and time interval from training completion with two exceptions. Females under 35 were more likely to opt for cesarean themselves ($p = 0.04$), and 42.9% of fellows under 35 interpreted the medical literature as supporting cesarean in all cases versus 4.2% of older colleagues ($p = 0.008$). Sixty-four of 78 (82.1%) respondents would find a randomized trial of planned vaginal versus planned cesarean delivery helpful in addressing the issue of patient choice cesarean. **Conclusion:** While Maine ACOG fellows are willing to perform patient choice cesarean, few prefer this delivery mode for themselves or their partners. A randomized trial of planned vaginal versus planned cesarean delivery is highly desired.

The Improved Role Of Fructose-1,6-Diphosphate On Nervous System Development Of Newborns With Hypoxic-Ischemic Encephalopathy

Zhao Xiaopeng, Liu Guosheng et al. **Maternal and Infant Hospital of Guangzhou, Guangdong Province, Peoples Republic of China**

Objective: To determine the effects of treatment with fructose 1,6-diphosphate (FDP) on the prognosis of neonates with hypoxic-ischemic encephalopathy (HIE).

Methods: 124 newborns suffering from HIE were randomly divided into two groups. The control group (n=62) received standard treatment and therapy. The FDP group (n=62) were also treated with continuous intravenous FDP (250mg/kg.d) drip. FDP treatment was begun within 48 hours after birth and continued for 7 days, in addition to standard therapy. In followup, NBNA was performed at 12~14 days and 26~28 days of age in all patients. Intelligence test was also performed at 3 and 6 months after birth.

Results: In those infants with moderate or severe HIE, NBNA score and mental development were better in the FDP group than in the control group at both time points ($P<0.05$, $P<0.01$).

Conclusion: Early treatment with FDP could markedly improve the prognosis of neonates with HIE.