Tissue Engineered Gas Exchange Device Using MicroElectro Mechanical Systems Technology Supports Alveolar Cell Line

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Purpose: Primary pulmonary hypertension and cystic adenomatoid malformation are types of lung disease encountered in infants. Ventilation strategies for premature neonates can result in chronic lung disease. Tissue engineering offers a potential solution via a live, functional engineered organ. A tissue engineered gas exchanger could provide oxygen and remove carbon dioxide in full-term infants with lung disease, and allow time for lung development in premature neonates. Fabricating large three-dimensional tissue has inherent challenges due to limited oxygen and nutrient diffusion. A novel approach has been implemented in our laboratory. MicroElectro Mechanical Systems technology enables construction of a scaffold of an intrinsic microvascular network from biocompatible polymer. This preformed vascular network can be seeded with cells and ultimately sustain growth and function of complex tissues.

Methods: The prototype device consisted of vascular and parenchymal chambers, separated by a membrane. A network of vascular channels was etched onto a silicon wafer. These silicon wafers were used for replica molding of poly(dimethyl siloxane) (PDMS), which served as the polymer scaffold. The parenchymal chamber was seeded with a mouse alveolar type II cell line (MLE-12). Culture medium flowed through the vascular chamber at 0.5 ml/hr. The cells were assessed for viability using Live/Dead Assay Kit at 65 hours post-seeding.

Results: After 65 hours, there were large areas of viable MLE-12 cells. The viability was similar to control cells seeded and incubated for 72 hours on a PDMS coated 12-well plate.

Conclusions: We have demonstrated viability of MLE-12 cells in a novel device designed to overcome oxygen and nutrient diffusion limitations. This is an important initial step in the development of a tissue-engineered gas exchange unit.

Insulin-like Growth Factor-1 Mediated Airway Remodeling in Oxidant Injury in Developing Lung

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Significance: Bronchial wall remodeling is a major morbidity component in oxidant injury in BPD and asthma. IGF-1 modulates repair processes after lung injury. Hypothesis: IGF-1 enhances collagen synthesis in developing lung fibroblasts leading to fibrosis through nuclear NF-kB-dependent transcription. Design: We studied the signaling pathway activated by IGF-1 in a human fetal lung fibroblast cell line (HFLF). We studied NF-kB dependent transcription by transfecting HFLFs with a NF-kB responsive promoter driving the luciferase gene and treating with IGF-1 (100ng/ml). Luciferase activity was measured by luminometer. We also exposed cells to the PI-3 kinase inhibitor (LY294002) or the Erk1/2 inhibitor (PD98059) one hr prior to stimulating with IGF-1. Western blots were probed with antibodies directed against phospho-AKT, AKT, phospho-IKKB, IKKB, Extra Cellular Signal-regulated Kinase (Erk1/2), and type I collagen. Blots were quantified by densitometry. Results: IGF-1 treatment significantly increased luciferase activity in transfected HFLFs. This was attenuated by PI-3 Kinase and MAP-Kinase inhibitors. Western blot analysis showed a significant increase in Phospho AKT with IGF-1 that was blocked by the PI-3 kinase inhibitor LY294002 ($P<0.05$) but not PD98059 suggesting that PI3 Kinase mediates IGF-1 activation of NF-kB. There was no significant change in IKkB phosphorylation in cellular protein or in p65, a DNA binding subunit of NF-kB in the nuclear extract. IGF-treated cells had a significant increase in collagen synthesis ($P< 0.05$). This was significantly inhibited by pre-treatment with LY294002. These studies show that IGF-1 cell signaling leading to collagen synthesis is mediated by PI3 Kinase acting through NF-kB in developing lung. We propose this is an important pathway in the response to oxygen injury in BPD.

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α2 Integrin Expression in Human and Mouse Lung Branching Morphogenesis and Aberrant Expression in Human Bronchopulmonary Sequestration (BPS) and Congenital Cystic Adenomatoid Malformation (CCAM): Potential Regulatory Role in Hoxb-5

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Integrins bind extracellular matrix molecules (ECM) and neighboring cells to mediate cellular signaling. Hox genes regulate integrin expression, but the relationship of Hox genes and integrins in lung development is unknown. Hoxb-5 helps regulate airway branching during lung development and is abnormally upregulated in mesoderm surrounding the aberrant airway branches of human BPS and CCAM. We hypothesized that 1) Hoxb-5 inhibition down regulates α2 integrin expression: and 2) α2 integrin expression is altered in human BPS and CCAM lesions. To test hypothesis (1), GD13.5 fetal mouse lung fibroblasts were cultured 72 hrs with Hoxb-5 specific siRNA to inhibit Hoxb-5 expression and α2 integrin measured by Western blot. To test hypothesis (2) α2 integrin protein was measured by Western blot in normal human lung (gestation 15 wk to term) and in BPS and CCAM tissue, using separate antibodies to the α2 integrin cytoplasmic region (Ab1) and the extracellular domain (Ab2). In Gd13.5 fetal mouse lung fibroblasts siRNA inhibition of Hoxb-5 decreased α2 integrin protein by 50%. In normal human lung, similar to Hoxb-5, α2 integrin was highest in the pseudoglandular and canalicular stages. In BPS and CCAM tissue Ab1 detected one α2 isoform at 150 kD that was 30% higher in CCAM. Ab2 detected a second α2 band at 130 kD that was upregulated by 82% in BPS and 62% in CCAM compared to age matched controls. We conclude that α2 integrin is a downstream target of Hoxb-5 in mouse and human lung development. In BPS and CCAM, Hoxb-5 upregulation may lead to transcription of previously undetected α2 splice variants with altered cytoplasmic signaling moieties resulting in aberrant α2/beta1 integrin signaling in these lesions.

Serial Neonatal Oral Motor Assessment Scale (NOMAS) as Measure of Feeding Readiness

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Objectives: To determine the inter-observer reliability of serial blinded NOMAS assessments as a measure of feeding readiness in preterm infants.

Study Design: Prospective observational study

Study Participants: 125 preterm infants (gestational age ranging from 23 to 36 weeks) admitted to the Neonatal Intensive Care Unit at Tufts-New England Medical Center were enrolled.

Methods: Enrollment occurred within 48 hours of eligibility review and underwent serial weekly videotaped non-nutritive and nutritive sucking periods, performed within a 72 hour window of the 0/7 week gestation. Concomitantly, the bedside nurse completed a questionnaire regarding the child's feeding status. The videos were identified by study identification number, and feeding evaluation number. These videos were later reviewed independently by blinded NOMAS trained individuals. Inter-rater reliability was calculated and validity through comparison to the bedside nursing assessment.

Results: Results of the NOMAS are normal, disorganized, or dysfunctional. Disorganized patterns reflect an abnormal rhythm and central nervous system immaturity. Dysfunctional patterns reflect abnormal motor movements and do not reflect immaturity. There are 28 features of tongue and jaw movements as part of the NOMAS assessment.

Preliminary results document inter-rater reliability of the feeding pattern by NOMAS assessment to be over 95% concordance on blinded, independent review. On specific feeding features, concordance is 88% to over 95%.

Conclusion: Feeing is critical to a newborn's ability to survive and indicates central nervous system integrity. Successful feeding is based on reflexive maturation at 34 weeks post-menstrual age (PMA). The preterm population is at particular risk for difficulty with oral feeding, which can contribute to prolonged hospitalization, increased medical costs, additional short-term morbidity, and may portend long-term problems with growth and development. Previously, identification of infants at risk for feeding difficulties has been hampered by the lack of a universally available assessment of feeding. Preliminary data suggests that the NOMAS is a reliable assessment tool and provides an objective, standardized, observational measure of infant's feeding maturation. This will provide a standardized measure by which to identify those preterm infants at risk for feeding difficulties.

TNFA -308 G>A Polymorphism Influences the TNF-α Response to Altered Vaginal Flora and Pregnancy Outcome

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OBJECTIVE: To investigate the association between a tumor necrosis factor-α (TNF-α) gene polymorphism, mid-trimester vaginal TNF-α levels, microbial flora, and pregnancy outcome.

METHODS: Vaginal samples from 203 women at 18-22 weeks' gestation were analyzed for microflora, TNFA-308 G>A polymorphism, and TNF-α concentration. Outcome data were subsequently obtained. RESULTS: TNFA -308A carriers with bacterial vaginosis (BV) had higher median TNF-α levels (10.94 pg/ml) than did TNFA-308A carriers without BV (1.77 pg/mL, P= .02) or TNFA-308G homozygotes with BV (1.72 pg/ml, P= 01). Among black, but not white or Hispanic women, TNFA -308A carriage was associated with preterm delivery (Odds Ratio= 17; 95% CI= 1.05 -917.25) and low birthweight (2954g vs. 3376g) (P= .02).

CONCLUSION: TNFA -308A carriage is associated with an exaggerated TNF- α response to BV-associated vaginal flora and, in black women, with spontaneous preterm delivery and low birthweight.

Mid Aortic Syndrome in a Premature Infant Presenting As Unexplained Hypertension and Necrotizing Enterocolitis (NEC)

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Background: Mid Aortic Syndrome (MAS) is an uncommon condition characterized by segmental narrowing of the proximal abdominal aorta and its major branches. It is usually diagnosed in young adults, but may rarely present in neonates. Early diagnosis and management of MAS is important to prevent complications. **Objective:** To highlight early signs and symptoms of MAS through a case presentation, and to discuss the diagnosis and management of this condition in newborns. **Case Presentation:** A female was born at 29 weeks gestation to a primigravid 28 year old. Sonogram 1 week prior showed severe polyhydramnios, fetal cardiomegaly with decreased function, and evolving fetal hydrops. Antenatal steroids and indomethacin tocolysis were administered, and amnioreduction performed. Delivery was by cesarean section due to worsening fetal cardiac contractility with a presumptive diagnosis of premature ductal closure following Indocin therapy. Apgar scores were 5, 7 and 8 at 1, 5 and 10 minutes of age. At delivery, birthweight was 1395 g. The baby had hepatosplenomegaly and was hydropic with moderate edema of the head and trunk. Chest and abdominal radiographs showed pleural effusion and ascites. Initial blood pressure (BP) was 73/42, mean 54. Dopamine and epinephrine were administered for poor myocardial function, however she subsequently developed systolic BPs >90. Pressors were discontinued and her hypertension treated initially with hydralazine, then Captopril. Echocardiogram on day 1 showed moderate PDA with left-to-right shunt and no evidence of cardiac anomaly or coarctation. A renal sonogram on day 2 showed right renal artery and vein thrombosis, both of which resolved by day 4. Based on her clinical course, a presumptive diagnosis of MAS involving the renal arteries was made on day 10. Over the subsequent weeks, her blood pressure remained difficult to control medically, and her creatinine gradually increased from 1.4 to 1.9 mg/dL. Contrast magnetic resonance angiogram/catheter studies could not be performed in view of the size of the baby and risk of thombosing critical vessels in such a small infant. Careful initial breast milk feedings were later changed to an elemental formula due to feeding intolerance. On DOL 29, the infant succumbed to fulminant NEC. Autopsy confirmed MAS with narrowing due to hypoplasia of the abdominal aorta from the celiac axis extending to the bifurcation of the common iliac arteries, narrowed superior mesenteric artery, narrowed left and right renal arteries; shrunken right kidney with hemorrhagic left kidney, and cardiomegaly. **Conclusion:** MAS should be included in the differential diagnosis of neonatal hypertension. The associated morbidity and mortality depend upon the extent of involvement of thoracic and abdominal vessels. Early diagnosis and management of MAS may reduce the risk of complications.

The Impact of Maternal Immune Status on the Development of Allergic Disease

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We have developed a murine model to elucidate the contribution of maternal immune status (Th1- vs. Th2-type immunity) on the development of allergic airway disease in offspring. In this model, female C57BL/6J mice were immunized to generate Th1-biased (OVA/CFA) or Th2-biased (OVA/Alum) immune responses to challenge with aerosolized ovalbumin (OVA/Aer). Females were then bred with nonsensitized C57BL/6J males and subjected to repeat OVA/Aer to induce Th1- or Th2-type immune responses during pregnancy. Offspring from both groups, and additional age and sex-matched controls (without exposure to OVA-sensitized mothers) were assessed for their ability to respond to OVA-induced allergic airway disease (homologous challenge) starting at 1 month of age. In separate experiments, the antigen specificity and "environmental" effects of exposure to Th1-biased immunity during pregnancy were assessed. Female mice were immunized, mated and challenged in a similar fashion to generate Th1-type inflammation to the heterologous antigen BSA during pregnancy (BSA/CFA; BSA/Aer). Offspring born to mothers with Th1-type immunity to BSA were then evaluated for their ability to respond to OVA-induced allergic challenge in postnatal life (heterologous challenge). Our results indicate that offspring of Th1-type immune mothers were more protected from the development of allergic airway disease than offspring of Th2-type immune mothers when challenged with homologous antigen in postnatal life. However, this protection appeared antigen-specific and did not apply towards novel antigens unrelated to maternal exposure. These results suggest that maternal immune status may be a crucial factor in determining the manner in which newborns respond to antigens in postnatal life. While Th1 immunity appears protective against the development of allergic disease, our data indicates that this protection is antigen restricted.

PLACENTAL INFLAMATION BETWEEN 23-28 WEEKS IS ASSOCIATED WITH A INCREASE IN PRO-INFLAMMATORY CYTOKINES WITHIN PLACENTAL STROMA

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Direct assessment of cytokine concentrations in the preterm placenta remains unexplored. We tested the hypothesis that the expression of pro- and anti-inflammatory cytokines within the chorionic stroma of placentas differs by placenta histology.

Thirty placental specimens were obtained immediately after consecutive delivery between 22 and 27 completed weeks for the indications of severe preeclampsia (PE), membrane rupture (pPROM) or preterm labor (PL). Under sterile conditions, the amnion overlying the disk was inspected to ensure that the chorion was not exposed. The amnion was then peeled away to expose the chorion. A 1cc piece of chorion and underlying tissue was cut, flash frozen and stored at -80OC.

The sample was thawed, and homogenized in PBS in a 1:10 dilution. The lysate was run in duplicate on the MesoScale Discovery multiplex platform. This platform simultaneously evaluates multiple analytes in a single 25 microliter sample. Confirmation of each analyte was obtained by standard ELISA.

Histology was evaluated in a blinded, structured exam performed by a research pathologist. Chorioamnionitis was defined as the presence of neutrophils within walls of the major chorionic vessels or umbilical cord. Given the non-normal distributions of cytokine concentrations, non-parametric regression was used to evaluate differences.

Histologic chorioamnionitis was present in 40% of the placentas but not present in any of the PE placentas. Chorioamnionitis was associated with increased concentrations of IL1beta, IL6, and IL8 ($p<0.01$ each). Non-significant increases in TNFalpha, and reductions in IL10 and IL1ra were observed. Both the concentration and rank ordering of the expression of each analyte was then confirmed by standard ELISA (correlation coefficients 0.89-0.99).

Restricting the analysis to non-inflamed placentas, PE placentas had reduced IL10 and IL1ra levels ($p<0.01$) compared to the other delivery indications. Levels of IL1beta, IL6, IL8 and TNFalpha were not significantly elevated.

Chorioamnionitis, as indicated by vascular inflammation, is accompanied by increased concentrations of pro-inflammatory cytokines in extremely preterm chorionic tissue. These results confirm the hypothesis that histologic inflammation is associated with inflammatory cytokinemia within the placenta. Reduced anti-inflammatory levels in the PE placentas suggest this may be a state of imbalance between pro- and anti- inflammation. This is the first multiplexed measurement of cytokine concentrations within a single specimen in this tissue source with validation by standard technique.

Does sepsis at birth predispose premature infants to subsequent episodes of sepsis?

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Introduction: A majority of sick and premature infants are treated with a course of antibiotics at birth based on a presumptive diagnosis of sepsis. The impact of this on subsequent episodes of sepsis is not well understood. The aims of this study were to describe the effect of sepsis at birth on subsequent episodes of sepsis in premature babies during NICU stay.

Methods: A total of 616 premature infants (≤ 36 wk GA at birth) were admitted to the NICU at the University of Connecticut Health Center between January 2004 and September 2005. Of these 546 premature infants who were suspected to have sepsis were included in the study and the remaining 70 premature infants who were never suspected to have sepsis were excluded. Antibiotic treatment was initiated based on maternal and birth history of risk factors for sepsis and the infants' clinical condition. Treatment was discontinued after 48-72 hours if risk of sepsis was considered low (no-sepsis group). Treatment was continued for 5-10 days based on a combination of history, clinical condition and laboratory data including microbial cultures (sepsis group). If sepsis occurred within first 72 hours after birth it was categorized into 'birth sepsis' group. Sepsis occurring 72 hours after birth was categorized as 'nosocomial sepsis'. Comparisons were made between infants who had birth sepsis (B) versus those who had nosocomial sepsis (N) or a combination of birth and nosocomial sepsis (B+N). The number of episodes of subsequent sepsis and characteristics of organisms isolated from cultures were analyzed.

Results: Of the 546 infants, 170 infants with sepsis at birth were treated with ≥ 5 days of antibiotics, remaining 376 infants were not.
Comparisons of the groups are shown in table below.

Variable	Babies treated with ≥5 days of antibiotics at birth 'N=170'		Babies not treated with ≥ 5 days of antibiotics at birth 'N=376'	
	Birth only N= 115(68%)	B+N N= 55 (32%)	Nosocomial (N) N= 39 (10%)	No sepsis N=337 (90%)
GA in wks (mean ± SD)	31.5 ± 4	27.8 ± 3	29.1 ± 3	32.6 ± 3
BW in gms (mean ± SD)	1873 ± 700	1067 ± 496	1245 ± 499	1954 ± 593
Male Sex	71(62%)	30 (55%)	15 (38%)	174 (52%)
Organisms identified	1 (0.8%)	32 (58%)	28 (72%)	NA
Gram negative	1	4	0	NA
Fungal	0	4	0	NA
Gram positive	0	24	28	NA

Treatment of infants with ≥ 5 days of antibiotics at birth was usually not based on positive microbial cultures. Infants treated for sepsis at birth were more likely to be treated for nosocomial sepsis than infants who had not been treated with ≥ 5 days of antibiotics (32% vs 10%; $p < 0.0001$). They were also 51%-74% more likely to have another episode of sepsis during their hospital stay ($P<0.0001$). During the period of the study, nosocomial fungal and gram negative sepsis occurred only in those infants who were treated for birth sepsis with ≥ 5 days of antibiotics. If infants had not been treated for sepsis with ≥ 5 days of antibiotics at birth then nosocomial sepsis was always due to gram positive organisms most commonly CONS.

Significance: Treatment of premature infants at birth with ≥ 5 days of antibiotics either is a surrogate for an unrecognized factor or is itself associated with increased incidence of nosocomial sepsis with increased predisposition for gram negative/Candida sepsis.

Determinants of Nursing Management of Oxygen Saturation in Extremely Premature Infants

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Background: Factors influencing nurse practice when managing oxygen saturation (SpO2) in infants <28 weeks gestation (extremely premature infants, EPIs) are not defined. **Objective:** To identify factors influencing nurse SpO2 practice in EPIs. **Design/Methods:** Nurses in US NICUs with neonatal-perinatal fellowships were surveyed in 2004. Data were collected re: stated upper and lower SpO2 values maintained in EPIs, and center and nurse characteristics that may influence practice. Factors influencing individual SpO2 practice were identified with hierarchical modeling (HM). **Results:** 2823 nurses (45% of eligible) in 59 NICUs (60% of eligible) responded. 68% of NICUs had a policy for SpO2 range in EPIs. In HM, presence of a SpO2 policy, center mean opinion among nurses re: best upper and lower SpO2 limits in EPIs, and individual opinion re: best limits were significantly associated with individual practice. Years NICU experience modestly increased low SpO2 practice. Presence of a policy reduced the individual opinion-practice slope. These 4 factors accounted for 81% of between-center and 36% of within-center variance in individual SpO2 upper practice, 96% of between-center and 52% of within-center variance for lower practice. **Conclusions:** Presence of a policy for SpO2 practice, nurse group opinion, and individual nurse opinion significantly influence the upper and lower limits of individual nurse SpO2 practice in EPIs. Lower SpO2 practice increases with experience. The presence of a policy reduces the influence of individual opinion on practice. Efforts to standardize nurse SpO2 practice in EPIs should address these factors. Important influences on SpO2 practice remain unidentified.

Determinants of upper end of SpO2 practice	Coefficient (S.E.)	P
Center mean high practice		
Adjusted overall high practice intercept	-2.73 (6.26)	-
Center mean opinion among nurses re: best high SpO2 practice	1.03 (0.07)	<.001
Effect on center mean high practice of having policy in place	-0.81 (0.19)	<.001
Individual opinion		
Mean individual opinion-practice slope for centers with no policy	0.60 (0.04)	<.001
Effect of having a policy upon mean individual opinion-practice slope	-0.19 (0.05)	.001
Determinants of lower end of SpO2 practice		
Center mean low practice		
Adjusted overall low practice intercept	3.98 (2.76)	-
Center mean opinion among nurses re: best low SpO2 practice	0.96 (0.03)	<.001
Individual opinion		
Mean individual opinion-practice slope for centers with no policy	0.68 (0.05)	<.001
Effect of having a policy upon mean individual opinion-practice slope	-0.16 (0.06)	.012
Years NICU nursing experience	0.012 (0.005)	.015

Table: Hierarchical models for determinants of individual nurse practice at upper and lower ends of SpO2 range in infants <28 weeks' gestation. Not significant: Admissions/year, # NICU beds, # nurses (center-level), primary shift worked, hours/week worked (nursing level).

Correlation of Random Urine Protein/Creatinine Ratio with 24-Hour Urine Protein in Women with Suspected Preeclampsia

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Objective: To evaluate the utility of random urine protein/creatinine ratio for diagnosis of significant proteinuria in women with suspected preeclampsia.

Study Design: A retrospective review was performed for 150 women with suspected preeclampsia at a single tertiary care center from 2/00-3/05. Women having a protein/creatinine ratio followed by a 24 hour urine for protein were included. Preeclampsia was defined by ACOG criteria. Significant proteinuria was defined as ≥ 300mg of protein in a 24-hour collection. Based on the 24-hour urine protein results, CMDT and SAS programs were used to generate the correlation coefficient, and the receiver operating characteristic curve. The sensitivity, specificity, positive and negative predictive values and the Youden index (best combination of sensitivity and specificity) were calculated for various cutoff points of the protein/creatinine ratio.

Results: 163 Samples were evaluated. The median maternal age was 32 years. Significant proteinuria was found in 70.5% of patients. The correlation coefficient was 0.753. The receiver operator characteristic analysis yielded an area under the curve of 0.88. The shoulder cut-off of 0.26 mg/mg gave a sensitivity and specificity of 74 and 90%, respectively; with a positive and negative predictive value of 94% and 59%, respectively. See Table

Conclusion: A protein/creatinine ratio of 0.26mg/mg is predictive of significant proteinuria in women with suspected preeclampsia. However, the high false negative rate (41%) necessitates the need for a 24-hour urine protein collection for definitive diagnosis.

Table

Cutoff (mg/mg)	Sensitivity	Specificity	PPV	NPV	Youden Index
0.19	0.80	0.81	0.91	0.63	0.61
0.26	0.74	0.90	0.94	0.59	0.63
0.30	0.67	0.90	0.94	0.53	0.57

Risk Factors for Postpartum Antihypertensive Medication Requirements in Severe Preeclampsia.

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Objective: To determine antepartum risk factors for postpartum antihypertensive medication use in women with severe preeclampsia.

Methods: A retrospective case control study was performed on patients who were diagnosed with severe preeclampsia between January 2000 and June 2004 at a single tertiary care center. Cases were patients who were discharged from the hospital on antihypertensive medications. Controls were patients who were discharged home on no antihypertensive medication. Antihypertensive medication was started postpartum when blood pressures were persistently >160/100mmHg. The following data were abstracted from maternal charts: age, parity, race, gestational age, body mass index (BMI), birth weight, antepartum medications, maternal medical conditions, smoking, HELLP syndrome, intrapartum antihypertensive requirements, and length of hospitalization after delivery. Risk factors were evaluated using multiple logistic regression.

Results: 241 patients had severe preeclampsia during the study period. 23 were excluded due to incomplete data. Of the remaining 218 there were 112 cases and 106 controls. There were no differences between the groups in age, race, gestational age, parity, BMI, or birth weight. After adjusting for confounding variables, only chronic HTN and intrapartum hydralazine use were associated with the need for postpartum antihypertensive medications (Table). A dose dependent association was seen with intrapartum hydralazine. HELLP syndrome was associated with a decreased likelihood for antihypertensive medications.

Conclusions: Patients with chronic hypertension and patients who required intrapartum hydralazine were more likely to require antihypertensive medications after discharge. These patients may benefit from starting antihypertensive medications in the early postpartum period.

	Odds Ratio	95% CI
<15 mg Hydralazine	2.26	1.19, 4.31
≥ 15mg Hydralazine	5.89	2.12, 16.3
Chronic HTN	7.45	3.01, 18.4
HELLP	0.36	0.15, 0.84

ErbB Ligand-specific Induction of Fetal Mouse Lung Type II Cell Proliferation and Differentiation

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Background: ErbB receptors (ErbB1, ErbB2, ErbB3, ErbB4) are important for development of several organs, including lung. The ErbB1 ligands epidermal growth factor (EGF) and transforming growth factor α (TGFα), and the ErbB4 ligand neuregulin (NRG) are crucial in the control of lung development. In developing systems, cell proliferation and cell differentiation are often in tension, a phenomenon we have reported in lung development. The relative effects of EGF, TGFα, and NRG on fetal lung type II cell proliferation and differentiation during development are unknown.

Objective: We hypothesized that EGF, TGFα, and NRG induce differential effects on fetal lung type II cell proliferation and differentiation, and that stimulation of one effect correlates to inhibition of the other.

Design/Method: Primary cultures of fetal mouse lung type II cells were prepared on d16, d17, and d18 of gestation (term=d19). Cells were grown in DMEM+20% stripped fetal calf serum to 80% confluence, serum starved for 3 hrs, then treated with EGF (10ng/ml), TGFα (10ng/ml), or NRG (33nM) for 24 hrs. Controls received DMEM only. During the 24hr treatment cells were also exposed to either ^{3}H-choline (to measure synthesis of surfactant DSPC) or ^{3}H-thymidine (to measure proliferation).

Results: The different ErbB ligands had gestation- and ligand-dependent effects on type II cell proliferation and DSPC synthesis (ANOVA $P<0.05$). Thymidine incorporation was strongly stimulated by EGF on d17 ($P<0.05$). No significant changes in proliferation were observed in d16 (range 85% to 116%) or in d18 (range 109% to 117%) cells. No treatment inhibited thymidine incorporation. EGF had minimal effect on DSPC synthesis except on d18. Both NRG and TGFα strongly stimulated DSPC synthesis on d17 and d18 (stimulation range 260%-285%; $P<0.05$ for each treatment). No treatment significantly affected d16 DSPC synthesis.

Conclusions: EGF, TGFα, and NRG did exhibit ligand-specific effects on proliferation and differentiation which were gestational age dependent. There was however no observable tension between cell proliferation and cell differentiation. We speculate that ErbB ligands strongly affect type II cell differentiation, while other factors may simultaneously reduce proliferative effects.

TITLE: Passive Second Stage of Labor and Assisted Delivery in Patients with Congenital Heart Disease

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BACKGROUND: Increasing numbers of women born with congenital heart disease (CHD) are surviving to childbearing age. In an effort to avoid the cardiac stresses that occur with maternal valsalva and exertion, many authorities have recommended a passive second stage of labor (SSL) and an assisted delivery (AD) for these patients. The impact of these interventions on obstetrical outcomes is unclear. **OBJECTIVE:** We hypothesized that a passive SSL and AD may be associated with an increased duration of SSL, rate of cesarean section, rate of 3rd or 4th degree laceration, rate of chorioamnionitis and postpartum hemorrhage (PPH). Given these expected associations, the decision to plan a passive SSL and AD should be individualized based upon the patients underlying congenital anomaly and functional cardiac status. **STUDY DESIGN:** A retrospective, non-randomized cohort study was performed on women with CHD who reached SSL with passive descent and intended AD. Between 1998-2004, Brigham and Women's Hospital served as the site of delivery for 90 pregnancies by 53 women with CHD. 48 eligible pregnancies underwent a trial of labor and reached the SSL. 37 of these patients did not valsalva during the SSL and constitute the study population. The remaining 11 patients whose cardiologists and perinatologists allowed them to valsalva during the SSL serve as the control population. Primary outcomes included duration of SSL, rates of cesarean section, chorioamnionitis, 3rd or 4th degree laceration, and estimated blood loss. Secondary outcomes include intrapartum or postpartum CHF, sustained arrhythmia, stroke, cardiac arrest and cardiac death. **RESULTS:** Of the 37 women in the study population, the average duration of SSL was 195.2 minutes; all 37 delivered vaginally. There were 5 episodes of PPH, seven 3rd or 4th degree lacerations, no episodes of chorioamnionitis, and one adverse cardiac outcome. Of the 11 women in the control group, the average duration of SSL measured 53.8 minutes; all 11 delivered vaginally. There were no episodes of PPH, one 3rd or 4th degree laceration, two episodes of chorioamnionitis, and no adverse cardiac outcomes. **CONCLUSIONS:** Though the significance of the differences in outcomes between the study and control groups is limited by size and lack of randomization, the lengthened duration of the second stage incurred with a passive SSL and assisted delivery does not appear to predispose mothers with CHD towards a significant increase in cesarean section, severe lacerations, PPH, chorioamnionitis, or adverse cardiac events.

Urinary Osteopontin Excretion is Low in Premature Infants at Risk for Nephrocalcinosis.

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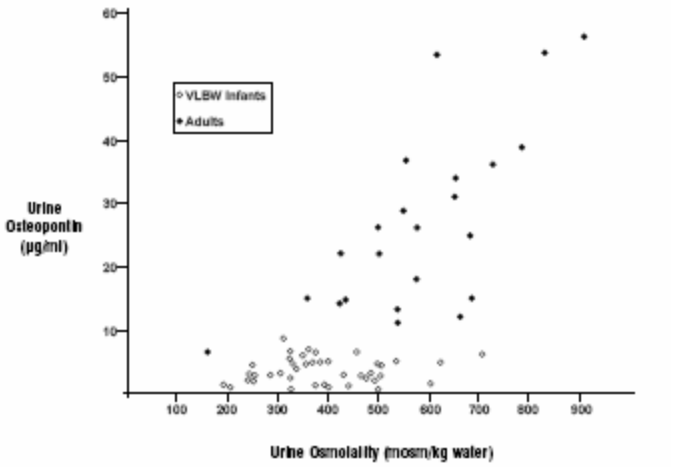
Nephrocalcinosis, the accumulation of calcium oxalate in the kidney, has been observed in 17-64% of premature infants and is associated with persistent abnormalities in glomerular and tubular function. Osteopontin (OPN) is an acidic calcium binding protein which is secreted by tubule cells and prevents the growth of calcium oxalate crystals in supersaturated urine. OPN deficient experimental animals develop renal calcium oxalate deposits and patients with abnormal OPN appear to be at increased risk for stone formation. No data are available regarding OPN excretion in premature infants who are at risk for nephrocalcinosis.

The objective of this work was to obtain preliminary estimates of OPN excretion in premature infants compared to healthy adults.

We measured urinary OPN in premature infants (n = 17, BW 1359 +/- 427 grams, GA 30.4 +/- 2.9 weeks) and healthy non stone-forming adults (n=11).

OPN was measured by ELISA.

The OPN concentration in urine from premature infants (3.42 +/-2.07 microgram/ml) was significantly lower than the urinary OPN concentration of healthy adults (24.16 +/- 17.87 microgram/ml))(p=0.007).



In adults, urinary osteopontin excretion correlated with urinary osmolality while there was no such correlation in premature infants. Similarly, urinary OPN excretion correlated with urinary calcium excretion in adults but not in VLBW infants, suggesting that adults are able to increase OPN excretion during periods of enhanced risk for calcium oxalate crystal formation, but premature infants are unable to respond in this way.

Conclusion: OPN excretion by premature infants is significantly lower than OPN excretion in healthy adults. Premature infants maintain low OPN excretion during periods of hypercalciuria.

Speculation: Developmental deficiency of OPN plays a role in the pathogenesis of nephrocalcinosis in premature infants.

MECHANICAL STRAIN ACTIVATES RHO AND INDUCES STRESS FIBER FORMATION IN FETAL LUNG TYPE II EPITHELIAL CELLS. Ophira Silbert*, Yulian Wang, Benjamin Maciejewski, Sunil K. Shaw, Juan Sanchez-Esteban. Department of Pediatrics, Women and Infants Hospital and Brown University, Providence, RI.

BACKGROUND: Mechanical forces are critical for normal fetal lung development. However, the mechanisms by which pulmonary cells sense and transduce mechanical signals are largely unknown. Actin cytoskeleton plays a key role in mechanotransduction by serving as a scaffold for protein-protein interactions. The Rho GTPases (Rho, Rac, Cdc42) are a family of proteins that regulate actin cytoskeleton organization and gene expression by integrating mechanical cues into specific signal transduction pathways. Its role in lung development is currently unknown.

OBJECTIVE: To define the role of the Rho family GTPases in actin cytoskeleton remodeling and fetal type II epithelial cell differentiation mediated by mechanical strain.

METHODS: Fetal rat pulmonary type II epithelial cells were isolated at E19 (term=E22) and cultured on laminin-coated silastic membranes. Cyclic elongation (5%, 60 cycles/min) was applied for varying intervals using the Flexercell FX-4000 Strain Unit to simulate mechanical forces in utero. Cells grown on nonstretched laminin-coated plates were used as controls. Activation of the Rho GTPases was analyzed by affinity precipitation assays. Actin distribution was directly visualized under the microscope using cells infected with adenovirus expressing GFP/actin and exposed to 5% continuous strain using the StageFlexer Jr apparatus. Also, ERK phosphorylation was assayed by western blot.

RESULTS: Mechanical strain maximally activated Rho after 15 min by 2.5-fold. However, neither Rac nor Cdc42 were stimulated by force. Real time fluorescence microscopy showed that mechanical strain reorganizes actin filaments into stress fibers in the direction of the force applied. The actin-polymerization inhibitor cytochalasin D and Rho inhibitors disrupted this organization. Cytochalasin D also significantly decreased strain-induced ERK phosphorylation, a key pathway in fetal type II cell differentiation mediated by force. Currently, we are investigating downstream activators of Rho and studying the role of this pathway in strain-induced type II cell differentiation.

CONCLUSIONS: Mechanical strain activates Rho and induces stress fiber formation in fetal type II epithelial cells. Our data also suggest that actin cytoskeleton remodeling may be important for strain-induced fetal type II cell differentiation.

THE CLINICAL UTILITY OF FETAL FIBRONECTIN TESTING IN TWIN GESTATIONS TO PREDICT PRETERM BIRTH

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Objective: To investigate the value of fetal fibronectin testing in twin gestations with symptoms of preterm labor, compared to its use in symptomatic singleton pregnancies.

Methods: This study consisted of a retrospective chart review from January 1, 2000 through June 30, 2004. All patients with twin gestations who presented with complaints of preterm labor, and had fetal fibronectin testing were reviewed. Also all singleton gestations that presented for evaluation of preterm labor and had fFN testing between Jan 1, 2000 and Dec 31, 2001 were reviewed. Only those patients with intact membranes, a cervical dilation less than 3cm, and a gestational age between 24.0 and 34.9 weeks at the time of testing were included. Deliveries induced for medical reasons such as preeclampsia or non reassuring fetal testing were excluded. Only the first test was included if a patient had fFN testing done more than once during the pregnancy. All samples were processed using the TLi system at Baystate Medical Center. The sensitivity, specificity, positive predictive value, and negative predictive value of fFN testing in singleton and twin gestations to predict delivery with 14 days of testing were calculated.

Results: A total of 433 singletons and 91 sets of twins met the inclusion criteria. Of the 28 sets of twins who tested positive for fFN, 21%(6) delivered in 14 days or less. The sensitivity and specificity for fFN testing in this population are respectively 75% and 74%. The positive predictive value was calculated to be 21% with a negative predictive value of 97%. A comparison with the singleton data is reviewed in the table below.

	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR +	LR -
Singletons	67	90	19	99	6.7	0.36
Twins	75	74	21	97	2.8	0.34

Delivered < 14 days From Testing

Conclusion: In twin gestations, fFN testing has similar negative predictive value as compared to singletons. The data suggests that in twins, fFN testing is as clinically useful for the evaluation preterm of labor as in singletons.

The Additive Prospective Risk of Intrauterine or Neonatal Death in Twins by Gestational Age: A Novel Method of Analysis.

Marie-Adele Sorel*, Amy Cohen, Thomas L. Beatty, Julian N. Robinson.

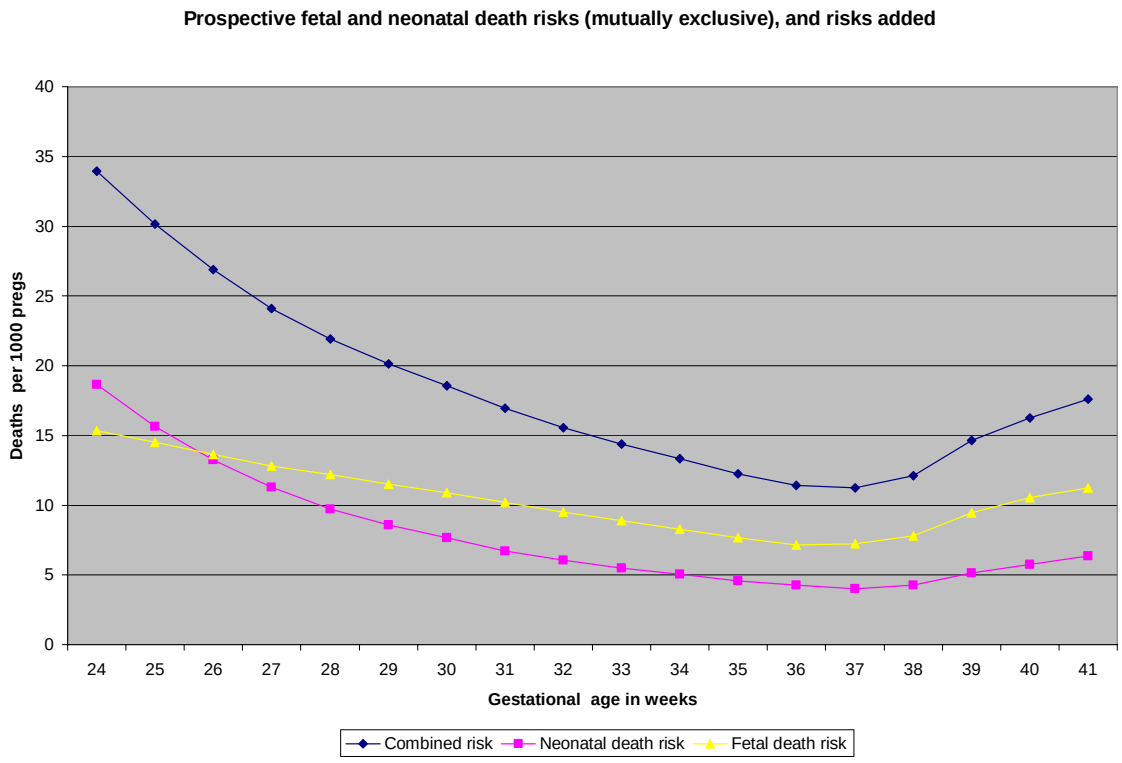
Objective

To determine the optimum time for delivery at which there is least risk of any fetal or any neonatal death for a twin pregnancy

Study Design

In the past we have published comparisons of the risk of intrauterine fetal death to the neonatal mortality rate by gestational age to derive the optimum gestational age for delivery of twins. As one statistic was a risk and one a rate, these statistics could not be used to assess additive risk of death. Here, we have calculated the prospective risk of neonatal death by gestational age so the additive prospective risk of either fetal or neonatal death can be demonstrated. The National Center for Health Statistics "Matched Multiple Birth Data" database for 1995 to 2000 was analyzed. Statistics were derived for 636,353 twin fetuses and 315,652 delivered twin pairs. The prospective risk of any fetal death within a twin pregnancy, the prospective risk of any neonatal death of a live born twin sibling and the additive risk of either were calculated by gestational age. These were represented graphically.

Results:



It can be seen from the graph, that for all of these data sets, the risk of death nadirs at 37 weeks and starts to increase at 38 weeks and continues to increase through term and beyond.

Conclusion: To minimize the risk of any fetal death in a twin pregnancy while pregnant, or any neonatal death after delivery, consideration should be given to elective twin delivery at 37 weeks of gestational age.

Tubal factor infertility is associated with increased risk of preterm birth

Alison M. Stuebe, Allison Cape, Mehmet Genc and Tom McElrath

Background: Several studies have documented an association between ART and poor obstetrical outcomes in singleton pregnancies. To date, no studies have assessed whether parameters of a couple's infertility diagnosis and treatment predict poor obstetrical outcomes.

Methods: We assessed obstetrical outcomes in singleton IVF pregnancies who underwent infertility treatment and subsequently delivered at Brigham and Women's Hospital between 1995 and 2000. Univariate analyses of tubal and male factor infertility were conducted, followed by multivariate logistic regression analysis.

Results: 526 women underwent IVF and delivered singleton infants during the study period. Of these, 246 carried a diagnosis of either tubal or male factor infertility, but not both. Among women with tubal factor infertility, 10.8 percent delivered at less than 32 weeks, compared with 1.2 percent of women with male factor infertility (p=0.004). We found a similar pattern for births at less than 28 weeks and for cerclage placement (see table). In multivariate logistic regression analysis including maternal age, parity, stimulated vs. non-stimulated cycle, and concurrent infertility diagnoses, tubal factor infertility remained strongly associated with birth at less then 32 weeks, OR 3.51 (95% CI 1.37-8.95) and birth at less than 28 weeks, OR 3.07 (95% CI 1.07-8.80).

Conclusions: Tubal factor infertility is associated with increased risk of preterm birth in singleton IVF pregnancies. Chronic inflammation from underlying tubal disease may increase the risk of preterm birth. Alternately, women with tubal factor infertility may have an exuberant response to inflammation that increases their risk of preterm birth. Further studies are needed to assess the mechanisms underlying this association.

Outcome	Tubal Factor Infertility % (n)	Male Factor Infertility % (n)	p (Fisher's exact)
N	111	135	
Birth < 32 weeks	10.8 (12)	2.2 (3)	0.004
Birth < 28 weeks	8.1 (9)	2.2 (3)	0.03
Cerclage	9.9 (11)	1.5 (2)	0.003

Sildenafil Citrate (Viagra), a selective phosphodiesterase type 5 inhibitor is a powerful pro- angiogenic agent.

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Introduction:

Myocardial ischemia is a major risk for infants undergoing early surgical interventions for congenital heart disease resulting in shock and low flow states. Sildenafil, a potent phosphodiesterase 5(PDE 5) inhibitor has been shown to induce cardio protection in Ischemia/Reperfusion (I/R) injury

Objective:

To test the hypothesis that Sildenafil induces preconditioning-like cardio protective effect by releasing angiogenic factors when used in therapeutic amount.

Methods

We examined the effect of Sildenafil using *ex-vivo* isolated rat heart model as well as using *in vitro* human coronary arteriolar endothelial cells (HCAEC) in culture. Rats were pretreated with Sildenafil (i.v) at doses ranging from 0.001 mg to 0.5 mg/kg body weight. After 60 min, isolated hearts were subjected to ischemia for 30 minutes followed by 2hr of reperfusion. HCAEC's were pretreated with two doses at 10 µM and 20 µM of Sildenafil subjected to 8hrs of hypoxia followed by 24 hrs of normoxia. Untreated controls were used for both models. Matrigel assay and western blot analyses were performed

Results

Sildenafil treated animals showed significant up regulation of Thioredoxin (TrX-1) (1.9 fold) along with Heme oxygenase (HO-1) (3 fold) expression. *In vitro* study with HCEAC's showed significantly increased tuberogenesis along with increased angiogenic factors like Angiopoeitin(ANG-1)(1.5 fold), Tie-2 (5-fold), along with Vascular Endothelial Growth Factor(VEGF)(2-Fold) and it's receptor KDR (4.5 fold) expression. Sildenafil along with HO-1 inhibitor, SnPP (10 µM) inhibited the expression of VEGF to 1 fold and Ang-1 to 0.7 fold as well as reduction in tuberogenesis.

Conclusions

The results demonstrated for the first time that within a narrow dose range, sildenafil could protect the heart from I/R injury probably through several important pathways. Further investigations are needed to understand the molecular mechanism(s) of sildenafil -induced cardioprotective effect, which would help in expanding the utility of this drug for pediatric cardiovascular diseases in addition to current postulated use in idiopathic and persistent pulmonary hypertension of newborn.

Differential Hoxa5, Hoxb4, and Hoxb6 protein expression in nitrofen-induced pulmonary hypoplasia (NT-PH) M V Volpe[1], K T Wang,[1] and M R Chinoy[2]. [1]Dept of Pediatrics, Tufts-New England Med Ctr, Boston, MA, and [2]Dept of Surgery, Hershey Med Ctr, Penn State Univ, Hershey, PA

Normal lung development requires precise temporal and spatial expression of master regulatory proteins including Hox proteins. In mouse and human lung, Hoxa5, Hoxb4, Hoxb5, and Hoxb6 are expressed in temporal and cell-specific patterns suggesting Hoxa5 involvement in alveolar development, Hoxb4 in proximal and distal lung development and Hoxb5 and Hoxb6 in bronchiolar branching. PH is characterized by abnormal lung morphogenesis with murine NT-PH mimicking the human condition. Previously we showed that in murine NT-PH, mesenchymal expression of Hoxb5 protein persists in late lung development in a pattern similar to earlier development but expression of Hoxa5, Hoxb4 and Hoxb6 in PH are unknown. **Objective:** Determine the expression profile and balance of Hoxa5, Hoxb4 and Hoxb6 proteins in normal mouse lung development and in NT-PH. We hypothesize that Hoxa5, Hoxb4 and Hoxb6 proteins would be modified in a pattern consistent with hypoplastic pulmonary development. **Methods:** CD-1 pregnant mice were untreated or gavaged with NT(25mg) on gestational day 8 (Gd8). Fetuses/neonates were sacrificed at Gd13.5, Gd15.5, Gd18.5 and P1(Neo). Hoxa5, Hoxb4 and Hoxb6 proteins were quantified in lungs from normal mice and compared with levels in NT-PH lungs by Western blot. **Results:** Across gestation, lungs from normal mice showed constant Hoxa5 protein levels, with a trend towards decreased Hoxb4 and increased Hoxb6. Compared to normal lungs, hypoplastic lungs had a 2 fold elevation in Hoxa5 protein levels at Gd18.5 and P1; a 70% increase in Hoxb4 at P1and a 50% decrease in Hoxb6 at all gestational ages. In NT-PH, there was 2 fold increased ratio of Hoxb4/Hoxb6 at all gestational ages; Hoxa5/ Hoxb6 at Gd18.5 and P1; and an increase in Hoxa5/Hoxb4 at Gd18.5(50%) and P1(20%). **Conclusions:** NT-PH is associated with altered protein levels of Hoxa5, Hoxb4 and Hoxb6. The relationship of these Hox proteins relative to each other may contribute to development of PH or be a compensatory mechanism to promote continued airway development and progression of lung maturation. Specifically, altered Hoxb4/Hoxb6 ratio may hinder the progression of lung maturation in PH, whereas altered Hoxa5 relative to Hoxb6 or Hoxb4 may compensate to promote progression of saccular development in this lung anomaly. Supported by HD 044784

Congenital Jejunal and Ileal Atresia—Natural prenatal sonographic history and association with newborn outcome.

Joseph R. Wax, MD[1], Thomas Hamilton, MD[2], Angelina Cartin[1], Janice Dudley, MSN, CPNP[2], Michael G. Pinette, MD[1], Jacquelyn Blackstone, DO[1]. [1]Division of Maternal-Fetal Medicine, Department of Ob/Gyn and [2]Division of Pediatric Surgery, Department of Surgery, Maine Medical Center, Portland, Maine.

Objective: To describe the prenatal sonographic features and natural course of congenital lejunal and ileal atresias, and correlate these findings with neonatal outcomes. **Methods:** We identified all neonates with surgically confirmed jejunal or ileal atresia that had prenatal ultrasound examinations in our center from 1/1/1995 – 4/1/2005 via prenatal ultrasound and pediatric surgical databases. Fetuses with cystic fibrosis or aneuploidy were excluded. Ultrasound reports and images were reviewed without knowledge of newborn outcomes for sonographic features of intestinal obstruction. Obstetrical data and neonatal outcomes were evaluated. **Results:** Fifteen of 25 (60%) offspring with atresias (10 jejunal, 4 ileal, 1 jejunoileal) had ultrasounds, of which 14 (93.3%) were consistent with an atresia. Findings, number of affected fetuses, and gestational age at recognition included echogenic bowel (FEB) (n=8) 21.3 ± 3.8 weeks (range 17.7-28.4), enlarged stomach (n=5) 27.5 ± 5.0 weeks (range 22.0-34.3), dilated bowel (n=13) 27.8 ± 5.8 weeks (range 18.3-35.9), and polyhydramnios (n=6) 33.3 ± 1.7 weeks (range 31.0-35.6). No fetus with ileal atresia had an enlarged stomach or polyhydramnios. In comparison, 5 (50%) neonates with jejunal atresia had enlarged stomachs (p=0.12) and 6 (60%) had polyhydramnios (p=0.15). Ultrasound findings were noted in 3 (75%) ileal and 10 (100%) jejunal atresias (p=0.36). Delivery occurred at 34.7 ± 3.6 weeks with a mean birthweight of 2430 ± 930 g. Six (40%) and 2 (13.3%) neonates had 1- and 5-minute Apgars <7, while one (5.5%) had a cord pH <7.20. Neonatal outcomes as measured by NICU days, age at surgery, TPN days, hospital days, and death were similar whether or not FEB, enlarged stomach, dilated bowel, or polyhydramnios were present. Likewise, neonatal outcomes did not vary by type of atresia (ileal vs jejunal) or time of diagnosis (prenatal vs. neonatal). **Conclusion:** Jejunal and ileal atresias demonstrate specific and predictable sonographic features that, allowing reliable prenatal diagnosis in most affected fetuses. Prenatal sonographic findings and timing of diagnosis did not affect neonatal outcome.

Nonmedical Fetal Ultrasound—Knowledge and opinions of obstetricians and radiologists.

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 evaluate knowledge and opinions regarding nonmedical fetal ultrasound (NMFU) in obstetricians (OB) and radiologists (R). **Methods:** A questionnaire was sent to all Maine Fellows of the American College of Obstetricians and Gynecologists (ACOG) practicing obstetrics and members of the Maine Society of Radiology in April 2005. The mailing received an institutional review board exemption. Data were analyzed using descriptive statistics, chi-square, or two-tailed t-test. **Results:** 64 of 111 (57.6%) OB and 29 of 100 (29.0%) R returned questionnaires (p=0.0000). 30 (46.9%) OB and 11 (37.9%) R noted local availability of NMFU, while 5 (7.8%) OB and 9 (31.0%) R did not know of availability (p=0.01). 9 (14.0%) OB and 3 (10.3%) R offered NMFU—none charged patients for NMFU. Among OB, 52 (81.2%), 24 (37.5%), 45 (70.3%), and 56 (87.5%) did not know if the American College of Radiology, ACOG, American Institute of Ultrasound in Medicine, or Food and Drug Administration (FDA) held positions on NMFU. Among R, 11 (37.9%), 27 (93.1%), 19 (65.5%), and 24 (82.7%) did not know if the organizations held positions. 3 (10.3%) R and 21 (34.4%) OB agreed that NMFU should be available to women at their own expense (p=0.01). 4 (13.8%) R and 21 (33.9%) OB agreed that NMFU should be available if the woman had a prior medical ultrasound (p=0.03). 1 (3.1%) R and 7 (11.1%) OB would write a prescription for a NMFU. More R than OB agreed that women might forego medical ultrasound after NMFU (62.1% vs 49.2%, p = 0.05), while more OB than R believed fetal anomalies would go undetected during NMFU (79.4% vs 62.1%, p=0.04). OB and R had concerns for false positive NMFU diagnoses (41.9%, 31.0%), false reassurance by NMFU (76.2%, 62.1%), poor imaging causing anxiety (39.7%, 51.7%), and lack of physician availability to review suspected abnormalities on NMFU (73.0%, 65.5%). Few OB and R believed NMFU providers should be disciplined by licensing boards (33.9%, 44.8%), excluded from society memberships (22.9%, 37.9%) or reported to the FDA (21.3%, 31.0%). **Conclusions:** Most OB and R are concerned about NMFU. Few OB and R provide NMFU, are aware of professional and regulatory bodies' NMFU position, or favor sanctions against those offering NMFU.

Pain associated with transabdominal chorionic villus sampling—Anticipated versus actual.

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Objective: to assess anticipated and actual pain associated with transabdominal chorionic villus sampling (TA CVS). **Methods:** Consecutive women undergoing TA CVS completed a preprocedure 10cm visual analog scale (VAS), where 0=no pain and 10=excruciating pain, to describe anticipated pain. After TA CVS, each patient completed an identical VAS to describe actual pain. All procedures were performed by the same operator, without local anesthetic, between 10 0/7 – 13 4/7 weeks, under real time ultrasound guidance, using freehand technique, and a single 20 gauge spinal needle. Sixteen subjects would provide 80% power at the 0.05 significance level to detect a 1.5 cm difference in anticipated versus actual pain. Data were analyzed using descriptive statistics and continuous variables were compared by the paired student t-test. **Results:** All procedures on the 16 participants were successful on the first attempt. No complications occurred. Anticipated pain was 5.1 (95% CI 3.5-6.7) ± 2.9 cm, range 0-10. Actual pain was 5.5 (95% CI 3.8-7.2) ± 3.2 cm, range 0.5-10 ($p = 0.43$). The difference between anticipated and actual pain was 1.4 (95% CI 0.6-2.2) ± 1.5 cm. When compared to anticipated pain, actual pain was less in 5 (31%), the same in 6 (38%), and greater in 5 (31%) patients. **Conclusions:** Anticipated and actual TA CVS-associated pain are similar and of moderate degree. These findings provide baseline data for patient counseling and interventional trials to decrease procedure-related pain.