Incidence, Mortality Rate and Associated Risk Factors for Developing Clinically Significant Pulmonary Hemorrhage in VLBW Infants.

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Background: Pulmonary hemorrhage (PH) complicates the hospital course of 1-5% of preterm infants. It markedly increases the risk of death or chronic lung disease with a reported mortality of approximately 50%. With recent advances in neonatal practice the mortality rate associated with PH may have changed.

Objective: To determine perinatal risk factors associated with PH in VLBW infants (<1500 g) born from 1990 through 2008 and detect any temporal change in incidence and mortality associated with PH.

Method: A retrospective study analysis of data in VLBW infants born from 1990 to 2008. We collected information on maternal and neonatal characteristics. The incidence and mortality rate were compared during two periods (Period I: 1990-1999, and period II: 2000-2008). Descriptive, comparative and regression analysis were performed. PH diagnosis was made based on clinical and radiographic criteria.

Results: Of 2281 patients studied, 87 developed PH (3.8%).The following factors were associated with an increased risk of developing PH: Lower GA, RDS, PDA, severe IVH, male sex, C/section, no antenatal steroids and lower 5 min. apgar score. In a multiple logistic regression analysis only GA, RDS, delivery by C/section, and low 5 min. apgar score remain significant. There was no change in the incidence of PH between the two periods (3.8% vs. 3.7%, p=0.385). There was a reduction in mortality associated with PH in period II (76% vs. 38%, p=0.001). There was a difference in PDA, antenatal steroid, and severe IVH between the two periods. After adjusting for these potential confounders, the mortality rate associated with PH remained lower in period II (table 1).

Conclusion: The main risk factors associated with PH are: Lower GA, RDS, PDA, and delivery by C/section. The mortality rate associated with PH has decreased significantly in this decade; however the incidence of PH has not changed. We are looking at specific treatment changes to explain this difference.

Table 1- Multivariate logistic regression of potential confounder associated with reduction in mortality

	OR	95% CI	P- value
Periods (II vs. I)	0.23	0.07-0.63	0.005
PDA	0.29	0.10-0.88	0.029
Antenatal steroid	1.38	0.48-4.00	0.553
Severe IVH	3.55	1.10-11.82	0.039

Transportable Enhanced Simulation Technologies for Pre-Implementation Limited Operations Testing in the Neonatal Intensive Care Unit (TESTPILOT-NICU)

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INTRODUCTION: Migration of a Neonatal Intensive Care Unit (NICU) to a new physical plant incurs many challenges, which are amplified by switching the culture of care to single family rooms from a bay type model. Healthcare delivery systems must be altered to maintain their efficiency and safety nets. Simulation offers a milieu to explore systems fallibility prior to moving patients.

METHODS: This observational study was conducted in the new adjacent 50,000 square foot facility, six to nine weeks prior to moving patients, amidst the fine-tuning of facilities and communication systems. Our aims were to identify vital staff orientation material, and to assess translation of existing processes while exposing staff to the new NICU. We hypothesized that 1) participants would find the sessions fruitful, and 2) numerous process gaps would be discovered, despite meticulous planning. Each simulation session balanced typical staff demand in six concurrent vignettes, calibrated to generate two to four concurrent high acuity situations. After extensive orientation, participants performed their usual duties in two 30-minute in situ simulations followed by facilitated debriefings.

RESULTS: Response to recruitment was robust; 96 participants from all specialties, with 1 to 35 years NICU experience, half of whom had never experienced simulation. Among the 85% who completed evaluations, the majority stated this experience would change their practice (Likert scale 0-5, score [SD] was 4.21 [0.73]) and would recommend simulation to their colleagues (4.84 [0.79]). Assimilating all feedback, we remodeled systems for recruiting bedside assistance and for code blue response. We recommended 35 specific changes for verbal and written communication protocols, and 64 other workflow changes. Fifteen safety issues were identified, as well as 38 other minor facilities and supplies issues. Areas of concern were raised for staffing (11), training (12), and the simulation itself (8).

DISCUSSION/CONCLUSIONS: Simulation is very effective for identifying process gaps prior to major institutional change. Most participants thought TESTPILOT positively affected patient care, and the ripple effect on workflow committees and staff orientation planning was remarkable. The extensive coordination required to implement such large scale simulations is well worth the benefit for systems refinement and patient safety.

Title: Peripartum Bacteremia in the Era of GBS Prophylaxis

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Background: Pregnant women are at risk for serious bacterial infection in the peripartum period. The microbiology

and clinical risk factors associated with peripartum maternal bacteremia have not been reviewed since the

widespread implementation of intrapartum antibiotic prophylaxis for maternal Group B Streptococcus (GBS)

colonization.

Methods: We identified all cases of maternal bacteremia occurring in the peripartum period (defined as seven days

before delivery to 30 days post-partum) in a large maternity center from 2000-2008. Chart review was performed to

determine the clinical factors associated with bacteremia.

Results: During the study period, 78,781 women delivered 81,376 live births. Peripartum bacteremia occurred in

170 women (incidence 2.2 cases per 1000 deliveries) accounting for 190 individual microbial isolates. The most

frequent individual bacterial isolates were E. coli (36.6% of isolates); enterococci (23.7%); and anaerobic species

(12.4%); GBS were isolated in only 8 cases (4.8%). Polymicrobial infections were found in 24 women. Persistent

bacteremia was noted in 6.5% of cases despite antibiotic therapy (range, 2-5 days). In five cases of maternal

bacteremia, the same bacterial species were isolated from infant blood cultures obtained immediately after

birth. Clinical diagnoses associated with peripartum bacteremia included endometritis (54.7%); chorioamnionitis

(25.3%); urosepsis (6.5%); and other or unknown (13.5%). Among women with endometritis, 75% delivered by

Cesarean section (RR 7.39, 95% CI 4.61, 11.84) and 40% delivered at less than 37 weeks gestation (RR 3.21, 95%

CI 2.42, 4.25). Severe complications of peripartum bacteremia were noted in 6% of women: one death occurred in a

woman with postpartum urosepsis; six women developed systemic inflammatory response syndrome; a total of six

women were admitted to the intensive care unit. Ileus developed in five women, one of whom required a colostomy

due to pseudo-obstruction resulting in intestinal perforation.

Conclusion: In the era of GBS prophylaxis, E.coli and enterococci are the most frequent bacteria isolated in

peripartum bacteremia; GBS accounted for less than 5% of cases. The relatively high incidence of bacteremia and

diverse isolates seen among febrile peripartum women demonstrates the importance of obtaining blood cultures in

this population.

Title: Post-cerclage ultrasound monitoring of cervical length for predicting gestational age at delivery

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Introduction: It is unclear from the literature whether following cervical lengths after cerclage placement is predictive of gestational age at delivery and thus a useful practice.

Methods: Women with singleton pregnancies and vaginally placed history indicated cerclages over the past 5 years at our institution were identified. Women who had at least two transvaginal ultrasounds between 20 & 30 weeks were included. A linear regression model was run to determine the association between gestational age at delivery, the initial cervical length and a change in cervical length of >5mm prior to 30 weeks.

Results: Complete information was available for 380 women. The mean GA for the first measurement was 21.8 weeks (SD=1.7) and for the last measurement was 28.1 weeks (SD=1.9). Results can be seen in Table 1. Women with a shorter initial cervical length delivered at an earlier gestational age for all categories however this was significant only in the <2.0cm category. Women who had cervical shortening greater than 5mm also delivered at earlier gestational ages regardless of the initial cervical length category. The combination of an initial cervical length of <2.0cm and cervical change was associated with the earliest gestational age at delivery.

Conclusion: These results confirm that, in a population of patients with a history indicated cerclage, cervical length <2cm at 21 weeks is associated with an earlier gestational age at delivery. More importantly, shortening in the cervix by more than 5mm is associated with an early mean gestational age at delivery for all length categories. Initial length and change in length may provide different perspectives on the duration of gestation in this population.

Table 1: Mean gestational age at delivery by initial cervical length, with and without change in cervical length prior to 30 weeks.

Category:	Change by >0.5cm or more	(N)	Mean GA at delivery	95% CI	p-value
1) 0 to 2.0	No	(60)	35.8	27.1, 41.8	0.041
	Yes	(22)	31.5	21.1, 39.4	<0.001
2) >2.0 to 3.0	No	(77)	37.3	28.7, 43.3	0.171
	Yes	(40)	35.9	25.8, 43.0	0.049
3) >3.0	No	(136)	38.5	29.9, 44.5	0.40
	Yes	(45)	36.4	26.6, 43.6	0.001

Title: Neonatal outcomes in 23-29 week neonates following indomethacin tocolysis

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Introduction: Earlier work has shown that indomethacin tocolysis can be associated with increased risks of intraventricular hemorrhage (IVH) and necrotizing enterocolitis (NEC) but with exposures much higher than the amount accrued in the 48 hour course to which this agent is typically limited in current practice. There is no data available regarding the frequency of spontaneous intestinal perforations (SIP). This study was designed to determine whether typical current obstetrical usage of indomethacin is associated with risks of IVH, NEC, SIP or persistent patent ductus arteriosus (PDA) in the less than 29 week neonate.

Methods: Infants less than 29 weeks gestation exposed to *in utero* indomethacin within four weeks of delivery were matched to infants not exposed by gestational age, exposure to betamethasone for 48 hours, rupture of membranes for 24 hours, and year of delivery (2003-2008). All babies were delivered for the reasons of preterm labor or preterm premature rupture of the membranes. Cox modeling was used, and a clustering analysis was performed to account for multiple gestations. This study had a power of >0.95 to detect a difference in NEC or in grade 2-4 IVH of the magnitude previously reported in the literature.

Results: 69 exposed infants were matched to 69 unexposed. There were no significant differences along the matched dimensions, exposure to one or multiple tocolytic agents, age, race, mode of delivery, multiples and chorioamnionitis. There was no significant difference between groups for IVH, NEC, PDA or SIP. This remained true when analyses were restricted to exposure within the 7 days (N=49 per group) and 48 hours (N=37 per group) preceding delivery. There was no trend to suggest an underpowered positive result for NEC or IVH however SIP showed a non-significant trend towards a harmful effect. In the entire cohort, PDA requiring surgical ligation also trended towards a harmful effect (HR 1.41, 95% CI 0.93, 2.14).

Conclusion: These results suggest that at current tocolytic dosing, indomethacin does not lead to IVH or NEC in newborns. Further research is needed to clarify the effects of current dosing of indomethacin on PDAs and with regards to risk of SIP.

Comparison of Cervical Ultrasound and Obstetrical Outcomes Between Abdominal Cerclages Placed Via Laparoscopy or Laparotomy Prior To Pregnancy and Laparotomy During Pregnancy

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Objective: Data is limited regarding the mode of placement of an abdominal cerclage. We compared the outcomes of abdominal cerclages placed using three different techniques in our center.

Study Design: We performed a retrospective case series of 38 patients with singleton pregnancies who had abdominal cerclages placed at BWH between 1999-2009, in one of three circumstances: 1) via (gravid) laparotomy during pregnancy, 2) laparotomy prior to pregnancy, or 3) laparoscopy prior to pregnancy. CL were then measured at 24 weeks and used for comparison. The primary outcome was delivery at 36 weeks since institutional protocol is for delivery of abdominal cerclage patients between 36-37 weeks prior to spontaneous labor. Ranksum, median regression, and Chi-square were used for significance testing.

Results: Table 1	Abdominal Cerclage Types and Outcomes			
	Gravid, Laparotomy	Laparotomy	Laparoscopy	p-value
N (%)	11 (29)	20 (52.6)	7 (18.4)	
Maternal Age	32 (30-36)	34 (31-35)	37 (35-40)	0.02
Gravidity	3 (4.5-5.5)	3.5 (2-4)	3.5 (2-4)	0.7
Parity	0 (0-0.5)	1 (0-1)	0.5 (0-1)	0.14
Median CL at 24 weeks (mm)	41 (0-47)	41 (38-45)	44 (43-45)	0.66
Funneling Incidence (%)	4 (36.3)	0 (0)	0 (0)	0.002
Delivery GA	37.3 (37-38)	38 (36.2-38.1)	37.2 (36-38.4)	0.58
Delivery <36 wks (%)	2 (67)	0 (0)	1 (33)	0.16

Conclusion: We did not detect differences in the gestational age at delivery or incidence of delivery prior to 36 weeks among the three abdominal cerclage categories. However, patients with abdominal cerclages placed during pregnancy had increased funneling and a trend toward increased delivery <36 weeks.

The Direct Effect of Non-Directional Counseling.

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Objective: To determine if patients' choice of diagnostic testing when screen positive with first trimester combined screening is affected by the source of their counseling.

Materials and Methods: A retrospective study including all women who had a positive first trimester combined screen (risk of Down's syndrome >1:230) between December 2003 and January 2009 who had their obstetric care at a large university hospital. We compared the patient management choices according to whether they were counseled by a general obstetrician, a genetic counselor, or a perinatologist. The data was analyzed using Fisher's exact test and logistic regression analysis controlling for the degree of estimated risk.

Results: Of the 726 screen positive patients, 220 (30.3%) were counseled by a general obstetrican alone, 413 (56.9%) were counseled by a genetic counselor (under the supervision of a perinatologist,), and 93 (12.8%) patients were counseled by a perinatologist alone. The table below outlines patient management according to the type of counselor. The rate of any prenatal diagnosis is remarkably similar among the three groups (62.2 to 64.6%). However the selection of CVS and amniocentesis varies significantly between the groups (Fisher's exact test, P<0.0001). Logistic regression analysis shows that compared to counseling by generalists, counseling by genetic counselors is more likely to result in CVS uptake (OR 8.67, 95% CI 3.42, 21.99), and even more so with perinatologists (OR 9.9.80; 95% CI 3.52, 27.2).

Conclusion: The preference of prenatal diagnostic procedure for patients who are screen positive for Down´s syndrome in the first trimester varies significantly according to whether they are counseled by a general obstetrician, genetic counselor, or perinatologist.

Patients' Management Choice by Counselor					
	Total	**Prenatal Diagnosis**	**No Action**	**CVS**	**Amniocentesis**
General Obstetrician	220	142 (65%)	78 (35%)	5 (2%)	137 (63%)
Genetic Counselor	413	257 (62%)	156 (38%)	80 (19%)	177 (43%)
Perinatologist	93	58 (62%)	35 (38%)	24 (26%)	34 (36%)

Who Wants What: Deconstructing Patient Reaction to Risk Ratios

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Objective: To determine the optimal screening technique based on uptake rates for prenatal diagnostic procedures.

Materials and Methods: A retrospective cohort study including all women who had a positive first trimester combined screen (risk of Down's syndrome >1:230), between December 2003 and January 2009 who had their obstetric care at a university hospital. Descriptive statistics and logistic regression with fractional polynomial modeling were used.

Results: Our study includes 728 women, 457 (63%) of whom chose prenatal diagnosis. Of the subjects, 108 chose CVS (15%) and 349 chose amniocentesis (48%). Stratifying the estimated risk of Down's syndrome, the choice of expectant management varies from 26 to 50%, rate of uptake of CVS varies from 3 to 33%, and rate for amniocentesis varies from 41 to 54% (see Table). Logistic regression polynomial modeling of the data demonstrates that there is a significant association between the estimated risk of Down's syndrome and the proportion of patients choosing CVS ($p < 0.001$), but this is not the case for those choosing amniocentesis ($p > 0.05$). Specifically, polynomial modeling demonstrates a 20% uptake of CVS for an individual risk of 1:50. Similarly, descriptive statistics demonstrate a rate of CVS of 33% for the group with a risk of 1:50 and above, but 10% or less for lower risk groups. Thus, as risk decreases, the uptake of CVS trends downward, while that of amniocentesis remains more constant.

Conclusion: A clear association exists between risk and the decision to undergo CVS, and demand for CVS sharply decreases with risk. Thus, it appears that sequential integrated screening with a cut-off of 1:50 is appropriate for our population. These findings may vary across populations and local analysis is recommended.

Choice of Prenatal Diagnosis by Estimated Risk.

Estimated Risk	n	No Action	CVS	Amniocentesis
1:2 - 1:50	204	51 (25%)	68 (33%)	85 (42%)
1:51 - 1:100	166	60 (36%)	16 (10%)	90 (54%)
1:101 - 1:150	139	56 (40%)	11 (8%)	72 (52%)
1:151 - 1:200	130	65 (50%)	11 (8%)	54 (42%)
1:201 - 1:230	89	38 (43%)	3 (3%)	48 (54%)

Duration of Second Stage of Labor at 31 to 36 weeks of Gestation and its Effect on Neonatal Outcomes

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Objective: To evaluate the duration of second stage of labor in normal preterm vaginal deliveries at 31 to 36 weeks of gestation and its effect on neonatal outcomes.

Study Design: We retrospectively reviewed a total of 71,671 deliveries between 2002 and 2007 from Baystate Medical Center, Springfield, MA, MedStar Health, Baltimore, MD and Maimonides Medical Center, Brooklyn, NY. Out of 6,177 live born, singleton, preterm births with cephalic presentation, after excluding gestational age ≤ 30 weeks (n=960), cesarean (n=1,654) and operative vaginal deliveries (n=111), missing (n=634) or invalid (<1 min or ≥ 480 min) duration (n=88), a sample of 2730 was used for analysis. Duration of second stage was calculated in minutes by subtracting the time of complete cervical dilatation from time of delivery. Duration of second stage was compared across strata of epidural analgesia, parity and induction using two samples Kolmogorov–Smirnov statistic. A multivariable logistic regression was performed to evaluate the association of second stage with admission in neonatal intensive care unit (nicu), one minute and five minute apgar score ≥ 7 and length of neonatal hospital stay ≥ 1 week using second stage duration as continuous exposure.

Results: The mean ± S.D. of second stage duration was 52.3 ± 67.7 min, with a median of 26 min. The distribution of duration of second stage stratified by parity is presented in Table 1. Women with epidural analgesia (56.7 ± 69.1 min) had an overall longer second stage than women without epidural (45.8 ± 65.3 min, P <0.0001). However, duration of second stage was unaffected by labor induction in any of the gestational weeks and consequently in the total sample (induced vs. non induced: 55.1 ± 71.8 vs. 51.1 ± 65.9, P=0.73). After controlling for significant confounders, duration of second stage was not significantly associated with nicu admission (beta estimate (β): -0.001, 95% Confidence Interval (CI): -0.004 to 0.002), length of neonatal hospital stay ≥ 1 week (β: -0.005, CI: -0.01, 0.0004), one minute (β: -0.001, CI: -0.003 to 0.0001) or five minute apgar score being ≥ 7 (β: -0.0003, CI: -0.007 to 0.006).

Conclusion: Our findings provide a framework for clinicians to evaluate the decisive period of second stage in normal preterm vaginal deliveries at 31 to 36 weeks.

Table 1. Distribution of duration of second stage of labor for each week of gestational age stratified by parity.

Gestational age (weeks)	n (%)	Second stage in min (mean ± S.D.)			P value*
		Total	nulliparous (n=1213)	multiparous (n=1517)	
31	85 (3.1)	44.2 ± 74.2	54.4 ± 90.1	29.4 ± 39.2	0.01
32	144 (5.3)	49.9 ± 67.7	51.9 ± 71.7	47.7 ± 63.2	0.24
33	225 (8.2)	42.9 ± 53.4	47.4 ± 50.2	38.4 ± 56.3	0.0009
34	420 (15.4)	47.8 ± 62.7	54.1 ± 53.2	41.5 ± 70.6	<0.0001
35	643 (23.5)	56.1 ± 66.5	72.1 ± 67.1	44.3 ± 63.6	<0.0001
36	1213 (44.4)	54.3 ± 71.7	77.5 ± 75.8	38.7 ± 64.2	<0.0001
Total	2730	52.3 ± 67.7	66.9 ± 69.5	40.7 ± 63.9	<0.0001

* *P value from two sample Kolmogorov-Smirnov test for the null hypothesis that two samples come from similar distribution*

Differentiation of Human Cord Blood-Derived CD34+ Hematopoietic Progenitor Cells into Respiratory Epithelial Cells *in vitro* and *in vivo*.

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Background. The early stages of bronchopulmonary dysplasia (BPD) are characterized by increased levels of alveolar epithelial cell death, which may play a critical role in the alveolar disruption seen in BPD. Stem cell-based therapies aimed at restoring injured alveolar epithelium may be a powerful therapeutic strategy for BPD. Umbilical cord blood (UCB) represents an attractive and readily available source of stem/progenitor cells. While UCB-derived mesenchymal stem cells have been shown to be capable of pulmonary engraftment, albeit with low efficiency, the potential role of more easily generated and better characterized UCB-derived CD34+ hematopoietic stem cells (HSCs) remains largely undetermined. We hypothesized that UCB-derived CD34+ HSCs can differentiate into and populate respiratory epithelium of newborn lungs. **Aim.** To determine the capacity of human UCB-derived HSCs to differentiate into respiratory epithelial cells *in vitro* and *in vivo*. **Methods.** Human UCB-derived CD34+ cells from uncomplicated term deliveries were prepared by immunomagnetic sorting and characterized by flow cytometry and immunohistochemistry. The HSCs were cultured in the presence of growth factors, including keratinocyte growth factor and retinoic acid or in specific air growth media for 1 to 3 weeks. Respiratory epithelium-specific mRNA expression was studied by qRT-PCR. In addition, UCB-derived HSCs (5×10^5/pup) were administered intranasally to newborn FVB/N mice (P4). Lungs were studied at post-transplantation (post-TPX) weeks 1-8 for the presence of human and respiratory epithelial protein expression. The presence of human cells was further assessed by FISH analysis using probes for human chromosomes. **Results.** In the presence of specialized growth media and/or specific growth factors (retinoic acid), UCB-HSCs were capable of expression of thyroid transcription factor-1 (TTF-1), cystic fibrosis trans-membrane conductance regulator (CFTR), surfactant protein C (SP-C) and Clara cell secretory protein (CCSP). At post-TPX week 8, human-derived cytokeratin- and E-cadherin-positive epithelial cells were readily identified, engrafted within the alveolar wall. The presence of human cells was confirmed by FISH analysis. Rare human UCB-derived cells exhibited SP-C immunoreactivity, suggestive of respiratory epithelial differentiation. **Conclusions.** Human cord blood derived CD34+ progenitor cells have the capability to differentiate into lung epithelium both *in vitro* and *in vivo*. Future studies will test the therapeutic potential of these easily obtainable cells in the context of neonatal lung injury.

Regulation of Age-Dependent Type II Cell EMT Behavior

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Background: Epithelial- Mesenchymal Transition (EMT) is a biological process by which epithelial cells undergo multiple biochemical changes that enable them to assume a mesenchymal cell phenotype. EMT can be activated in association with tissue repair and remodeling after injury in multiple organs including the lung. In the adult lung EMT is associated with progression of fibrosis. In contrast, fetal tissue is capable of wound repair without scarring or fibrosis. We previously have shown that MLE12 cells, similar to primary adult type II cells, undergo EMT after transforming growth factor (TGF)β1 and to a lesser degree epidermal growth factor (EGF) treatment, resulting in the expression of mesenchymal cell phenotype. In contrast, fetal rat 21d type II cells did not undergo EMT in response to those treatments.

Objective: We hypothesize that ErbB receptors play a significant role in the age-related regulation of EMT.

Design/Methods: Isolated fetal d21 rat type II cells (>95% pure) were pretreated with cis-OH-proline to eliminate remaining fibroblasts, followed by a 5-day treatment of 2.5 ng/ml TGF-β1, 10ng/ml EGF, or the combination of both. Cells were harvested after 7d in culture for Western Blot analysis. MLE-12 cells were used as an adult lung epithelial cell model and treated similarly.

Results: TGFβ1 treatment induced phosphorylation and protein content of ErbB2 receptor in MLE12 cells. In fetal rat type II cells, TGFβ1 induced a decrease in ErbB2 phosphorylation. This effect was less modest in EGF-treated cells. The results of EGF treatment are in agreement with the less prominent EMT induction seen after EGF stimulation in both cell types.

Conclusions: These data suggest that regulation of ErbB receptor expression has a mechanistic role in the age-related induction of EMT. Further analyses are required to fully understand the function of ErbB receptor regulation in TGFβ1-induced EMT in type II cells.

MECHANICAL STRETCH RELEASES CYTOKINES AND MATRIX-METALLOPROTEINASES IN CULTURED FETAL MOUSE LUNG FIBROBLASTS

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BACKGROUND: Mechanical ventilation plays a central role in the pathogenesis of bronchopulmonary dysplasia (BPD). However, the molecular mechanisms and contribution of different lung cells to the inflammatory response present in BPD are not fully understood. We have previously shown that cyclic stretch of cultured fetal rat type II epithelial cells releases inflammatory cytokines and that IL-10 has a protective role.

OBJECTIVE: In these studies, using a murine model, we investigated the response of fetal lung fibroblasts to injury mediated by overstretching.

DESIGN/METHODS: Fetal mouse lung fibroblasts and type II cells were isolated at E18 of gestation (saccular stage) and cultured on silastic membranes precoated with fibronectin. Monolayers were then exposed to 20% cyclic stretch to simulate lung injury, using the Flexercell Strain Apparatus. Unstretched cells were used as controls. Release of cytokines and matrix-metalloproteinases (MMP) into the supernatant were analyzed by ELISA and Zymogram gel analysis, respectively.

RESULTS: 20% cyclic stretch of fetal fibroblasts increased KC and MIP-2 (murine homologues of the human IL-8) by 2- and 5-fold, respectively and decreased release of IL-10 by 50%, when compared to unstretched samples. In addition, mechanical stretch increased MMP-2 and MMP-9 into the supernatant by 2-fold. Currently, we are investigating whether administration of IL-10 prior to stretch affects the release of chemokines and MMP. In contrast, mechanical stretch of fetal type II epithelial cells did not change release of IL-1β, KC, MIP-2, MCP-1, TFNa, IFNg, MMP-2 or MMP-9 in the supernatant and modestly increased IL-6 and RANTES.

CONCLUSION: Our studies suggest distinctive roles for type II cells and fibroblasts in lung injury secondary to overstretching. Lung fibroblasts may play a critical role in the inflammatory response and extracellular matrix remodeling after injury mediated by mechanical stretch.

MECHANICAL STRETCH DECREASES IL-10 AND ITS RECEPTORS IN CULTURED FETAL LUNG TYPE II EPITHELIAL CELLS

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BACKGROUND: Although the mechanisms by which mechanical ventilation promotes bronchopulmonary dysplasia are not fully understood, an imbalance between pro- and anti-inflammatory cytokines seems to play a central role; in particular, the inability to maintain optimal IL-10 levels in response to an inflammatory injury. Previous studies from our laboratory showed that mechanical stretch of fetal rat type II cells decreased IL-10 production and the administration of IL-10 prior to stretch protected these cells from injury.

OBJECTIVE: Our primary aim was to analyze the mechanisms for inadequate IL10 function in fetal type II cells exposed to mechanical stretch.

DESIGN/METHODS: Fetal mouse lung type II cells were isolated at different gestational ages and cultured on silastic membranes precoated with fibronectin. Monolayers were then exposed to 20% cyclic stretch to simulate lung injury, using the Flexercell Strain Apparatus. Unstretched cells were used as controls. IL-10 release into the supernatant was analyzed by ELISA. IL-10 receptors gene expression was studied by real-time PCR. IL-10 signaling proteins were investigated by Western blot using phospho-specific antibodies.

RESULTS: 20% cyclic stretch of E18 type II cells for 24 hours decreased IL-10 in the supernatant by 58% compared to controls (p<0.02). Next, we investigated IL-10 receptors gene expression during different stages of development (E16-E18) and found an increase with gestational age. Furthermore, mechanical stretch reduced IL10R1 gene expression by 60%, and IL10R2 expression by 42% when compared to controls (p<0.0001). Finally, we analyzed receptor-associated kinases and found that mechanical stretch decreased phosphorylation of JAK1and TYK2 when compared to static samples.

CONCLUSION: Our studies suggest that IL-10 expression is developmentally regulated. IL-10 receptor gene expression and associated kinases are decreased following mechanical stretch. We speculate that the combination of both factors may explain the reduced ability for fetal type II cells to mount an anti-inflammatory response after lung injury mediated by mechanical stretch.

Stretch-Induced Differentiation Of Fetal Type II Epithelial Cells Requires An Intact EGFR

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Background. The ErbB receptors family (ErbB1, ErbB2, ErbB3 and ErbB4) plays an important role in lung development. Previous studies from our laboratory showed that stretch-induced differentiation of fetal type II epithelial cell is mediated via the EGFR (ErbB1)-ERK pathway.

Objective. To investigate whether other members of the ErbB family participates in stretch-induced differentiation.

Design/Methods. Whole fetal lung and type II cells were isolated from wild-type and EGFR (-/-) mice on E17-19 of gestation. ErbB receptors abundance and ERK phosphorylation were investigated by Western blot. Fetal type II cell differentiation was assessed by surfactant protein C (SP-C) mRNA and protein levels. Monolayers were exposed to 5% cyclic stretch to simulate fetal breathing movements, using the Flexercell Strain Apparatus.

Results. In the whole fetal lung of EGFR (-/-) mice and as expected, no ErbB1 receptor protein expression was detected. ErbB2 and ErbB3 increased by 1.6 fold and 4.5 fold, respectively, when compared to wild-type mice. In contrast, ErbB4 and SP-C protein decreased by 46% and 95%. In type II cells isolated from knockout mice, ErbB2 increased by 30% whereas ErbB3 and ErbB4 decreased by 38% and 24%, respectively. As expected, in type II cells isolated from wild-type mice, mechanical stretch activated ERK and increased SP-C mRNA and protein levels. In contrast, opposite results were observed in stretched cells isolated from knockout mice.

Conclusions. Despite some compensatory mechanisms in mice lacking the ErbB1 receptor, our preliminary data suggest that stretch-induced type II cell differentiation is almost exclusively mediated via the EGFR.

The Developing Microbiota: Mapping the Microbiome in Health and in Disease in Very Low Birth Weight Infants

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*in training

Background: Serious complications of prematurity, including sepsis, may be attributable to intestinal bacterial translocation. An understanding of colonization patterns as they relate to antibiotics and feeding – specifically composition, route of administration, and timing of feeds - may pave the way for future interventions to reduce the risk of bacterial translocation/sepsis in this high risk population. In this secondary study, part of a larger pilot study, we will investigate the clinical importance of early versus late enteral feedings (<4 days versus >4 days) as they relate to the developing microbiome.

Objectives: To explore the development of intestinal microflora in very low birth weight infants using novel mapping techniques (454 pyrosequencing) and relate colonization patterns to timing of enteral feeds.

Methods: Prospective study of 6 VLBW infants. DNA was extracted from weekly stool samples and 16s rDNA was amplified followed by high throughput DNA 454 pryosequencing to identify bacterial composition.

Results: We obtained 324651 sequences from 25 serial fecal samples from the 6 VLBW subjects. 2 subjects had sepsis: blood cultures positive for *C albicans* and *S aureus* (subject 1, week 4) and *S marcescens* (subject 4, week 4). Pyrosequencing of 16S rDNA genes demonstrated that diversity varied within each subject over time and between subjects and differed in neonates with sepsis and prolonged antibiotics. Subjects receiving prolonged antibiotics had preponderance (>80%) Proteobacteria. Neonates with documented sepsis had reduced diversity during the period of infection and antibiotic administration. The role of timing of initiation of feedings (early versus late) is being evaluated.

Conclusions: These results suggest that empiric, prolonged broad spectrum antibiotics profoundly decrease gut microbial diversity and promote preponderance of pathogens that may increase the risk of sepsis. Early feeding may alter colonization patterns and subsequently sepsis risk in this population.

RCT on the Effect of Massage on Methadone Exposed Infants

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Background: There has not been a study on the effect of massage for infants withdrawing from methadone exposure although massage improved weight gain and behavior for preterm infants.

Objectives: To see the utility of massage for neonates withdrawing from methadone exposure,

Methods: Infants born at 35-36 weeks are enrolled as preterm(PT) or full term(FT:37-42 weeks) at 3 sites. Infants Requiring morphine to captured dose are given Phenobarbital loading of 10 mg/kg twice, and then are randomized for clinical trial either to massage +SC(M) or standard care(SC) alone once a day and 5 times a week till weaning of Morphine. Phenobarbital was maintained at 2.5 mg/kg twice a day. Data are analyzed on LOS, NAS scores, the rate of decrease on Morphine dose and NICU Network Neurobehavioral Scale(NNNS). Statistical methods are general linear modeling, survival analysis and hierarchical linear modeling.

Results: The completed study has N=73. 1. Demographics: both groups are comparable in numbers of male, GA, BW, Apgars, 2 NAS scores before Morphine, maternal Methadone dose, age, parity, education level, smoking and other drug use except cocaine. 2. Overall LOS was shorter for M group (P=0.04) due to PT with covariates of Methadone dose, benzodiazepine and smoking. 3. The rate of decrease on Morphine was faster for PT-M Group (P=0.004). 4. There was no difference between M and SC in FT on NNNS summary scores with covariates on sites. Morphine and Phenobarbital improved quality of care, hypertonicity and excitability and regulation but not stress/abstinence in FT. 5. M improved attention in PT only after 5 massage sessions.

Conclusions: In PT, M shortened LOS. In FT, LOS were comparable among M and SC. LOS was not related to maternal Methadone dose. The rate of decrease on Morphine was more rapid for PT M group than SC. There was no difference among FT groups. There was no difference on NNNS scores among M and SC in FT. M improved attention in PT only. M affected PT and FT differently on some of NNNS summary scores.

ANGIOGENIC MARKERS IN SPONTANEOUS VS. ART PREGNANCIES

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OBJECTIVE: Pregnancies conceived through assisted reproductive technology (ART) are at an increased risk of pre-eclampsia, which may in part be due to abnormalities in early placentation and angiogenesis. Our study investigates whether serum angiogenic factors are different in spontaneous as compared to ART pregnancies.

STUDY DESIGN: A random sample of 341 singleton pregnancies (41 ART, 300 spontaneous) was selected from the analysis of hematologic based biomarkers and pregnancy outcomes study to compare sFlt and PlGF levels. Univariate analyses were conducted to examine means by ART status, using F-tests and Fisher´s exact tests, and the distribution of independent variables was examined with chi-square tests. Multivariable longitudinal linear regressions were conducted to test the association between serum angiogenic factors and ART status, including confounders (age, race, BMI, smoking, pre-eclampsia). The data was right-skewed, thus log-transformations were evaluated. We were powered to detect a 30% difference in sFlt levels (Power: 0.8).

RESULTS: As expected, sFlt and PlGF levels increased throughout gestation for both groups. ART pregnancies had lower levels of sFlt at <15 and 16-20 weeks and higher levels at 24-28 and 34-38 weeks (Figure 1). ART pregnancies had lower levels of PlGF at all gestational ages. These differences were not significant. There was also no statistically significant difference between the trend in either sFlt (p=0.46) or PlGF (p=0.94) levels when controlling for significant confounders.

CONCLUSION: Though the increased rate of pre-eclampsia in ART pregnancies may in part by accounted for by abnormal angiogenesis, we did not find this reflected in serum markers, which were not significantly altered in ART pregnancies.

Trends in sFlt Levels by ART Status
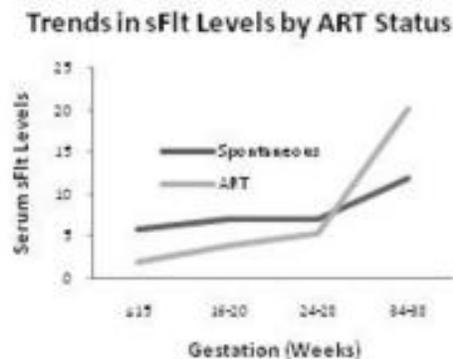
Androgen Inhibits TACE-Mediated Components of Fetal Type II Cell Surfactant Synthesis. Lucia D. Pham, Sana Mujahid, Sandy L. Murray, MaryAnn V. Volpe, Heber C. Nielsen. Tufts Medical Center, Boston MA.

Background: TACE is a member of the ADAM family of sheddase proteins. It is expressed in fetal fibroblasts and type II cells (T2) of gestational ages E16-E18. TACE activation in T2 cells stimulates surfactant synthesis. Exposure to dihydrotestosterone (DHT) throughout lung development delays fetal lung maturation, increasing the risk of respiratory morbidity in the newborn. However, the effects of androgen on TACE activity in lung development are still unknown.

Objective: We hypothesized that fetal exposure to androgen during lung development inhibits TACE activity, leading to T2 cells maturational delay.

Design/ Methods: Time pregnant mice were implanted with DHT pellets (2mg/day) on E11 (term = 19 days). Primary fetal lung T2 cells cultures were prepared from E17 control and DHT- implanted mice and grown with/without DHT (10^{-8}M). AT 80% confluence cells were serum starved for 3 hours, followed by no treatment (control), EGF (10ng/ml), NRG (3.3nM), TGFα (10ng/ml), PMA (TACE activator,100ng/ml), IC3 (TACE inhibitor, 50nM) or PMA+IC3 for 24 hrs. Surfactant production was measured as ^{3}H-choline incorporation into disaturated phosphatidylcholine (DSPC) and by qRT-PCR measurements of SP-B, SP-C, fatty acid synthase (FASN) and ABCA3 mRNAs. Western blots were used to determine levels of TACE, FASN, and ABCA3 protein.

Results: In control cells DSPC synthesis was stimulated ($p<0.001$) by EGF (240% of control), NRG (260%), TGFα (230%), and PMA (198%) but was reduced to less than control in the presence of TACE inhibitor (IC3 and PMA+IC3). SP-B mRNA expression was increased significantly ($p<0.05$) with exposure to EGF (350%), NRG (200%), TGFα (260%), and PMA (190%), while SP-C was increased significantly ($p<0.05$) with EGF (280%), TGFα (230%), and PMA (245%). In DHT-exposed cells neither DSPC synthesis nor SP-B, SP-C, FASN or ABCA3 were affected by any treatments. Comparing control and DHT- exposed cells, DHT significantly prevented the effects of EGF, NRG, TGFα and PMA on DSPC synthesis and on SP-B, SP-C, FASN, and ABCA3 mRNA.

Conclusions: Treatment with PMA (TACE activator) stimulated maturation of fetal T2 cells. However, DHT exposure inhibited the effects of EGF, NRG, TGFα and of PMA-activation of TACE T2 cell maturation. We conclude that androgen blocks TACE activity important for T2 cell maturation.

The Role of Betamethasone and Fetal Lung Maturity Testing in Late Preterm Cesarean Deliveries

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Objective: Respiratory morbidity (RM) remains one of the leading causes of neonatal intensive care (NICU) admissions in late preterm infants, especially in those undergoing cesarean delivery (CD). Recent evidence suggests antenatal betamethasone (BMZ) administration may reduce respiratory complications. Our objective was to evaluate the benefit of either BMZ or assessment of fetal lung maturity (FLM) when planning CD between 34 and 37 weeks.

Study Design: IRB approval was obtained for a retrospective chart review of all CD performed between 34+0 and 36+6 weeks gestation between January 2005 and March 2009. Maternal and neonatal records were analyzed for maternal demographic variables, delivery indications, amniocentesis, BMZ, gestational age at delivery and neonatal hospital course. RM included need for supplemental oxygen, intubation, surfactant or the diagnosis of TTN, PPHN, or RDS. Student's t-test was used for statistical analysis.

Results: 77 maternal/neonatal pairs were identified. Indications for CD included placenta previa, abnormal antenatal testing, placental abruption, rupture of membranes and malpresentation. The average GA at delivery was 35.7 ±0.9 weeks, with an overall RM rate of 36%. 50 patients (65%) had no FLM testing. Of those, 16 patients (32%) empirically received BMZ prior to delivery. 44% of those infants had RM (mean GA at delivery 35.2±0.7 weeks). There was a similar rate of RM in those not receiving empiric BMZ (44%, mean GA 35.1±0.8, p=0.8). 27 patients (35%) underwent assessment of FLM by amniocentesis prior to delivery. 12 (44%) had documented lung maturity; however, 4 of those infants (33%) had RM (mean GA 36.3 ± 0.5 weeks). 15 (56%) patients had an immature FLM. 10 then received BMZ prior to delivery. None of these infants had RM (mean GA at delivery 35.7±0.6 weeks). 5 did not receive BMZ, 2 of which had RM. GA was similar in all groups except those who had an immature FLM were younger than those with a mature FLM (35.6 ±.8 vs 36.6 ±0.5 weeks, p=0.01).

Conclusion: In our population, those patients undergoing indicated cesarean delivery prior to labor between 34 and 37 weeks gestation had a high rate of neonatal respiratory morbidity. This rate persisted even in those with mature FLM testing. Respiratory morbidity was low in those with a documented immature FLM who subsequently received BMZ. In our patients without FLM testing, empiric BMZ administration did not decrease rates of respiratory morbidity. It does not appear that routine use of BMZ in the late preterm infant will decrease overall respiratory morbidity.

Knock-down of Presenilin-1 (PSEN-1) Blocks ErbB4-Regulated Surfactant Synthesis in MLE-12 Cells

Anika Ritzkat*[1,2], Ekaterini Pringa, PhD[1], Melissa Dere[3], Cristina Scapin, PhD[1], Oya Guengoeze[1,2], MaryAnn V Volpe, MD[1], Christiane EL Dammann, MD[1,2] and Heber C Nielsen, MD[1,2,3]. [1]Tufts Medical Center, Boston, MA, United States; [2]Hanover Medical School, Hanover, Niedersachsen, Germany and [3]Tufts School of Medicine, Boston, MA, United States.

Background: ErbB4 receptor signaling is critical for type II cell maturation and surfactant synthesis. We reported that ErbB4 knock down inhibits surfactant protein production. The specific signaling pathway through which this is mediated is not known. A novel ErbB4 signaling mechanism involves membrane cleavage of ErbB4 by the secretase enzyme complex, followed by nuclear transport of the intracellular cleavage product. We have also shown that expression of PSEN-1, the active enzyme component of the secretase complex, is strongly increased in late gestational age fetal mouse alveolar type II cells.

Objective: We hypothesized that ErbB4 control of surfactant synthesis requires the activity of PSEN-1.

Design/Methods: We used pre-designed siRNAs (Applied Biosystems) targeting three separate regions of the PSEN-1 mRNA to knock down PSEN-1. We studied the effect of knock down on SP-B and SP-C mRNA levels using MLE-12 cells. First, to optimize conditions, cells were transfected with cocktails containing different levels of equimolar amounts of the three PSEN-1 siRNA sequences using Dharmafect 2 (Dharmacon) as the transfection reagent. Then RT-PCR was used to measure SP-B and SP-C mRNA with beta-actin as an internal standard. Results were expressed as the % change after PSEN-1 knockdown of scrambled siRNA-treated controls.

Results: Results: Optimum knock down (88% after 48 hrs) was achieved by a cocktail containing 5 nmoles of each sequence. Data for surfactant protein mRNA are the averages of two experiments, each done in triplicate. PSEN-1 knock down reduced mRNA for both SP-B (67% of scrambled control) and SP-C (61% of scrambled control). Treatment with scrambled siRNA or Dharmafect alone did not significantly affect SP-B or SB-C mRNA compared to untreated controls.

Conclusions: These data support the hypothesis that PSEN-1 activity is a crucial component of ErbB4 signaling for regulation of SP-B and SP-C gene expression in MLE-12 type II cells. Interpreted in the context of our previous studies, these data support a model of ErbB4 signal transduction involving nuclear localization of the intracellular ErbB4 fragment for regulation of surfactant protein synthesis.

Oxygen Saturation (SpO$_2$) Values Documented by Neonatal ICU Nurses vs Oximeter Recordings

TL Ruiz[1]*, JM Trzaski[2,3]*, DW Sink[2,3]* and JI Hagadorn[2,3]. [1]Health Fellows Program, Trinity Coll, Hartford, CT; [2]Neonatology, CT Child Med Ctr, Hartford, CT and [3]Pediatrics, Univ of CT Med Sch, Farmington, CT

Background: Continuous pulse oximetry is used by NICU nurses to document SpO$_2$ values for VLBW infants. **Objective:** To determine if SpO$_2$ values recorded by nurses differ significantly from actual oximeter saturations in VLBW infants. **Methods:** VLBW infants admitted from 1/08-6/08 had oximeter data collected prospectively every 2 sec for their NICU stay. Data were analyzed in two groups. Grp A consisted of one tenth of patient care hrs selected randomly regardless of respiratory support. Grp B included all hrs on supplemental oxygen (supO$_2$). SpO$_2$ values recorded hourly by nurses were obtained by chart review for all hrs in both groups. Proportions of data recorded by nurse and oximeter at each SpO$_2$ value, in target range (85-93%), and hyperoxemic range ($\geq$98%) were compared using concordance correlation (r_c) and goodness-of-fit X^2. **Results:** 30441 oximetry data hrs were collected from 24 infants (bwt 991±297 g, GA 27.6±2.2 wk) cared for by 87 nurses. Grp A consisted of 2862 hrs; Grp B contained 6511 hrs. In Grp A, proportion of data at each SpO$_2$ value recorded by nurses correlated well with the overall oximeter values (r_c = 0.989, p<0.001, Figure). Proportion of nurse charted SpO$_2$ values 90-96% was higher than oximeter (0.46 vs 0.41, p<0.001), while proportion 80-90% was lower (0.03 vs 0.07, p<0.001). In Grp B, compared to oximeter, nurses overrecorded SpO$_2$ values in target range for infants on HFOV and those <28 wks PMA, and underrecorded SpO$_2$ $\geq$98% at all PMAs (Table). **Conclusions:** Nurse recorded SpO$_2$ values reflected the overall SpO$_2$ experience of VLBW infants in this cohort as recorded by oximeter. Statistically and clinically important differences were found between frequency of SpO$_2$ values recorded by nurse and oximeter for target and hyperoxemic ranges for important subgroups of VLBW infants on supO$_2$. Primary electronic oximeter data are preferable when assessing achievement of SpO$_2$ goals in VLBW infants on supO$_2$.

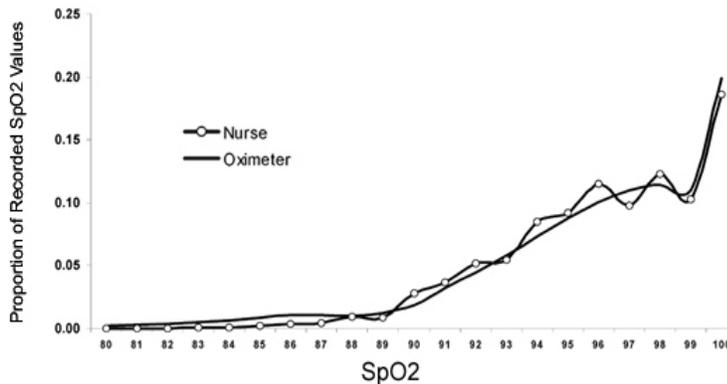Figure: Proportion of SpO2 values recorded by nurses vs oximeters for randomly selected patient care hours (Group A).

Table: Proportion of SpO2 values recorded by nurses vs oximeters in target range (85-93%) and hyperoxemic range ($\geq$98%), for all patient care hours on supO2 (Group B), subgrouped by PMA (wks) and respiratory support.

	Target Range			Hyperoxemic		
	Nurse	Oximeter	p	Nurse	Oximeter	p
<28	0.414	0.326	<0.001	0.115	0.148	0.017
28-31	0.300	0.263	<0.001	0.160	0.199	<0.001
32-35	0.160	0.170	0.212	0.283	0.320	<0.001
$\geq$36	0.019	0.026	0.104	0.810	0.831	0.027
HFOV	0.537	0.430	<0.001	0.027	0.038	0.256
SIMV	0.299	0.264	0.016	0.171	0.203	0.015
CPAP	0.293	0.257	<0.001	0.172	0.212	<0.001
NC	0.065	0.080	0.003	0.554	0.585	0.001

Title: Changing Oxygen Exposure (0.4 FiO$_2$ vs Room Air): Effects on Airway Branching, Hox Protein Expression and Mesenchymal Cell Fate

*F Silfa Mazara, MD[1,2], T Vong[1], I Larsson[1,2], H C Nielsen, MD[1,2] and M V Volpe, MD[1,2]. [1]Floating Hospital at Tufts Medical Center, Boston, MA, United States and [2]Tufts School of Medicine, Boston, MA, United States.

Background: Lung morphogenesis is adversely affected even by modest oxygen (FiO$_2$ $\leq$0.4), particularly in 23-26 wks gestation infants who are born when Hox proteins Hoxb5 and Hoxa5 play key roles in airway and alveolar morphogenesis. The effect of modest O$_2$ levels on these Hox proteins and on lung morphogenesis and cell fate is unknown.
Objective: Hypotheses: (1) Modest O$_2$-induced lung injury affects Hoxb5 and Hoxa5 protein expression altering cell fate and arresting lung morphogenesis; and (2) Recovery in room air (RA) partially reverses these changes and reestablishes airway growth and cell fate.
Design/Methods: Human fetal lung fibroblasts (HLF cells) and E14 whole fetal mouse lungs (after 48hrs culture) were assigned to RA (0.21 FiO$_2$), O$_2$ (0.4 FiO$_2$ 48 or 72h) or O$_2$RA (O$_2$ 24,48h/RA 24, 48h). Lung growth was measured as surface area (ΔSA). After culture, cells and lungs were processed for Hoxb5, Hoxa5, PECAM, α-actin, caspase 3, and PCNA imunohistochemistry and/or Western blot. Morphometry (point counts) determined changes in lung structure. **Results: HLF cells:** O$_2$ led to decreased Hoxb5 (p<0.001 O$_2$ vs RA), decreased PECAM staining and disorganized α-actin. O$_2$ RA had intermediate Hoxb5 levels. PCNA and α-actin protein levels followed this pattern. Hoxa5 nuclear localization was more intense after O$_2$ exposure but protein levels were unchanged . **Whole lung explants:** O$_2$ Gp had regressed airway branching and growth vs RA ($\downarrow$ ΔSA, p<0.005) but O$_2$RA Gp had new airway branches at explant periphery and $\uparrow$$\Delta$SA (p=0.01, O$_2$RA vs O$_2$). O$_2$ Gp had decreased Hoxb5 and increased Hoxa5 in mesenchyme with decreased PECAM around immature airways, increased peribronchial α-actin and mesenchymal and epithelial caspase 3. With RA recovery, Hoxb5 and PECAM localization was reestablished around new airway branches, α-actin increased around distal airways and caspase 3 was decreased. Morphometry showed $\downarrow$airway and epithelial cell volume (p$\leq$0.04) and $\uparrow$mesenchymal volume (p<0.01) in O$_2$Gp. This was partially reversed in O$_2$RA Gp.
Conclusions: Modest O$_2$ caused structural and cellular changes that were partially reversed by return to RA. Our data suggest that balanced expression of Hoxb5 and Hoxa5 contributes to regulation of endothelial cell fate and lung microvascular development. We speculate that altered Hoxb5 and Hoxa5 expression contributes mechanistically to O$_2$-induced lung injury in preterm infants. Support: HD044784, HL37930, Ikaria, AAP.

Abstract Title: Comparing Efficacy of Natural Surfactants in Preterms with Respiratory Distress Syndrome: A Systematic Review and Meta–analysis

*Singh N, Hawley K, Vishwanathan K (Dartmouth Hitchcock Medical Center, Lebanon NH)

Background: Natural Surfactants have been established to improve clinical outcome in preterm with RDS for last two decade. However, it is unclear whether significant differences in clinical outcome exit among the available natural surfactants.

Objective: To assess the effectiveness of Poractant alfa versus Beractant and Calfactant on respiratory outcomes in preterm with respiratory distress syndrome (RDS).

Methods: We searched the major electronic databases including MEDLINE, relevant conference proceedings, and reference lists of articles (from January 1980 to October 2009) for RCT's comparing Poractant alfa with either or both Beractant and Calfactant in preterm (<37 week) with RDS requiring intubation and surfactant treatment.

Main results: Four published RCTs and one unpublished RCT comparing Poractant alfa and Beractant included in the meta-analysis; no trial found comparing Poractant alfa and Calfactant. Preterm treated with Poractant alfa had 13% lower BPD rate (RR: 0.87 [0.67,1.13], p=0.29) , 42% lower mortality (RR: 0.58 [0.32,1.05] p=0.07) and decreased oxygen and ventilator support requirement. Statistically significant difference favouring Poractant alfa was noted in reducing need for redosing (RR: 0.52 [0.42, 0.65], p<0.001) and mortality (p=0.009) with higher initial dose. No difference was noted for outcomes related to other complications of prematurity.

Conclusion: The weight of evidence favors Poractant alfa by significantly reducing the need for redosing and mortality compared to Beractant.

Title: Anemia as a risk factor for Necrotizing Enterocolitis in Very Low Birth Weight Infants

Rachana Singh, MD[1,2], Paul F. Visintainer, PhD[1], Ivan D. Frantz III, MD[2,3], Bhavesh L. Shah, MD[1], Kathleen M. Meyer, MD[1], Sarah A. Favila[3*], Meredith S. Thomas[3] and David H. Kent, MD[2,3]. [1]Baystate Children's Hospital, Springfield, MA, United States; [2]Tufts CTSI, Boston, MA, United States and [3]Tufts Medical Center, Boston, MA, United States.

Background: Necrotizing enterocolitis (NEC) incidence in very low birth weight (VLBW) infants admitted to NICUs is 7-13% with mortality rates of 10-44%. All VLBW infants develop physiologic anemia which is aggravated by iatrogenic blood loss. Animal studies suggest that anemia may increase the risk of NEC.

Objective: To assess the effect of anemia on the risk of NEC in VLBW infants.

Design/Methods: This is a retrospective case- control study of VLBW infants diagnosed with NEC Stage 2a or greater (n=111), admitted to NICUs at Baystate Children's Hospital and Tufts Medical Center between Jan 2000 and Dec 2008. Each case had 2 gestational age (GA,± 1 week) and birth date (± 2 weeks) matched controls (n =222). The lowest hematocrit (Hct) for cases and controls was recorded for 96 hours prior to day of diagnosis for the case. Demographic and clinical data were collected. Statistical analyses included paired t-test, chi-squared analysis and linear regression to create propensity-like scores for the level of Hct and the final multiple conditional logistic regression model.

Results: The two groups had similar mean GA (Cases 26.±9 2.5wks, Controls 27.2 ±2.3 wks; p-value 0.21) and birth weight (Cases 970 ±336 gm; controls 1026 ±309 gms, p-value 0.14). After controlling for other factors, including a propensity-like score for Hct, a lower Hct was associated with increased risk of NEC [OR 1.11, p-value =0.007] Fig 1. Other factors associated with higher NEC risk in a multivariate model included hypotension, patent ductus arteriosus, presence of central venous lines and breast milk feeds with additives, while PPROM, abnormal end diastolic flow and iron therapy were associated with lower risk.

Conclusions: We conclude that anemia increases the risk of developing NEC in VLBW infants and this risk increases as anemia worsens. The protective effects of PPROM, abnormal end diastolic flow and iron therapy need to be further evaluated.

Title: Do Red Blood Cell Transfusions increase the risk of Necrotizing Enterocolitis in VLBW Infants?

Rachana Singh, MD[1,2], Paul F. Visintainer, PhD[1], Ivan D. Frantz III, MD[2,3], Bhavesh L. Shah, MD[1], Kathleen M. Meyer, MD[1], Sarah A. Favila[3], Meredith S. Thomas[3] [*] and David M. Kent, MD[2,3]. [1]Baystate Children's Hospital, Springfield, MA; [2]Tufts CTSI, Boston, MA and [3]Tufts Medical Center, Boston, MA.

Background: Red Blood Cell (RBC) transfusions have been implicated in the onset of Necrotizing Enterocolitis (NEC) in very low birth weight (VLBW) infants, especially in stable growing infants. There are some animal studies supporting this but a clear relationship has not been established for human infants

Objective: To assess the relationship between RBC transfusion and NEC in VLBW infants

Design/Methods: This is a retrospective case-control study of VLBW infants diagnosed with NEC Stage 2a or greater (n=111), admitted to NICUs at Baystate Children's Hospital and Tufts Medical Center between Jan 2000 and Dec 2008. Each case had 2 gestational age (GA ±1 week) and birth date (2± weeks) matched controls (n =222). The total number of transfusions during the NICU stay, lowest hematocrit (Hct) and RBC transfusion for cases and controls 96 hours prior to day of diagnosis for the case were recorded. Demographic and clinical data were collected. Statistical analyses included paired t-test, chi- squared analysis and logistic regression to create propensity scores for transfusion and the final multiple conditional logistic regression models.

Results: Cases and controls had similar mean GA (Cases 26.9 ±2.51wks, Controls 27.2± 2.32 wks; p-value 0.21) and birth weight (Cases 969.65 ±335.96 gm; controls 1026.2± 308.98 gms, p-value 0.14). Clinical correlates of VLBW infants, excluding Hct, did not discriminate propensity for transfusion (propensity OR = 1.06, p-value =0.75). In unadjusted analysis, cases were more likely to be transfused within 96 hrs than controls (crude OR =3.63, p=value 0.001). When adjusting transfusion for severity of anemia alone, treatment increases the risk (OR 2.42, p-value=0.005), but controlling for Hct & other clinical factors, attenuated the association of RBC and NEC (OR= 1.86, p-value=0.19). An inverse relationship between Hct and NEC risk stayed significant (OR=0.92,p-value=0.02) . There was no evidence of interaction between Hct and RBC transfusion.

Conclusions: While RBC transfusions may increase the risk of development of NEC, this association no longer reached statistical significance after controlling for a "transfusion propensity" within a multivariable model also including Hct and other important clinical factors. Experimental studies may be needed to tease out the potential influence of transfusions on the development of NEC.

Forty Percent Achievement of the Oxygen Saturation (SpO2) Target Range 85 - 93% is Associated with Improved Retinopathy of Prematurity Outcomes

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Background: Targeting lower SpO_2 to avoid hyperoxemia in very low birth weight (VLBW) infants is associated with reduced ROP. The percent target achievement needed to improve outcomes is unknown. **Objective:** To describe percent achievement of SpO_2 goals and incidence of oxygen-related outcomes before and after NICU SpO_2 policy adjustment. **Design/Methods:** We implemented the Oxygen With Love (OWL) program in a level 3 NICU to improve ROP outcomes. Incidence of ROP, ROP requiring laser, and other oxygen-related NICU outcomes were documented for inborn VLBW infants pre- and postOWL implementation. Continuous oximeter data from birth to 36 wk postmenstrual age were downloaded for a subset of infants in each cohort, with oxygen use recorded hourly, and proportion time on supplemental oxygen with SpO_2 values 85-93% (target), $\geq$ 98%, and <80% were determined. NICU outcomes and oximetry data pre and postOWL were compared. **Results:** NICU outcomes were assessed for 120 preOWL VLBW infants, of whom 14 had oximetry data collected. Of 33 postOWL infants, 30 had oximeter data. Demographics in the cohorts were similar (Table). PostOWL SpO_2 target achievement improved from 28.9 to 40.8% and hyperoxemia reduced from 26 to 8.9% of time on supplemental oxygen (Figure), with reduced ROP. For the postOWL cohort, proportion of total time, both on and off supplemental oxygen, with SpO_2<80% was higher (4.3 vs 1.3%, p<0.01, not shown in figure). **Conclusions:** OWL implementation resulted in an increase to 40% achievement of SpO_2 target range (85-93%) and decrease in hyperoxemia to <10% while on supplemental oxygen and was associated with significantly improved ROP. This is the first study to document change in SpO_2 target achievement, hyperoxemia and hypoxemia in VLBW infants after policy adjustment, and relate the changes to NICU outcomes. These results may help guide NICUs in reducing ROP through changes in oxygen management. Further research is needed to study the effect of these changes on long term outcomes.
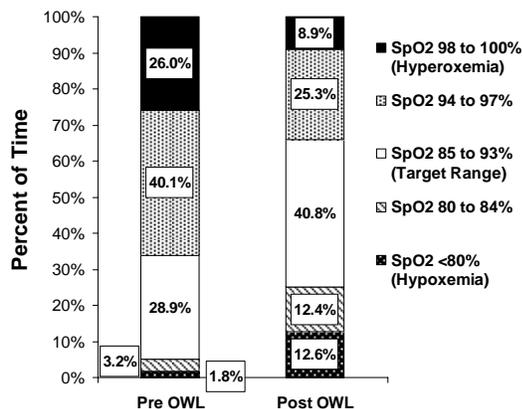
Table: Infant Characteristics and Outcomes

	Pre OWL (n = 120)	Post OWL (n = 33)	p
Birth weight, g	1074 ± 286	1096 ± 267	0.67
Gestational age, wk	28.5 ± 2.7	28.5 ± 2.5	0.90
Male, n (%)	72 (60)	19 (58)	0.84
ANS, n (%)	97 (81)	29 (88)	0.30
ROP, n (%)	40 (33)	5 (15)	0.02
ROP Surgery, n (%)	13 (11)	0 (0)	0.06
Oxygen at 36 wks, n (%)	20 (17)	6 (18)	0.84
PDA Ligation, n (%)	10 (8)	6 (18)	0.17
NEC, n (%)	7 (6)	3 (9)	0.54
LOS, days	74.8 ± 36.5	67.6 ± 31.5	0.28

ANS: antenatal steroids; ROP: retinopathy of prematurity; PDA: patent ductus arteriosus; NEC: necrotizing enterocolitis; LOS: length of NICU stay

Proteinuria In The Uncomplicated Twin Pregnancy
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Objective
We have observed that women with twin pregnancies appear to have higher rates of proteinuria without accompanying hypertension than do those with singletons. Protein to creatinine ratios (p:c ratio) in excess of 0.19 predict proteinuria greater than 300mg in a 24 hour collection. We compare rates of high p:c ratios in non-preeclamptic singleton and twin pregnancies in order to better understand normal protein excretion in twins.

Study design
A sequential sample of 102 (51 twins, 51 singletons) healthy patients without preeclampsia, gestational diabetes, intrauterine growth restriction, history of premature delivery or other medical comorbidities were selected from the Predictors of Preeclampsia Study to compare protein-to-creatinine ratio by fetal number. Samples were collected between 34 and 38 weeks gestation, and a clinically significant high p:c ratio was defined as greater than 0.19. Non-parametric statistical comparisons and logistic regression were used for analysis.

Results
Women with twin pregnancies were significantly more likely to have protein to creatinine ratios greater than 0.19 (p=0.003), and median p:c ratio was significantly higher in twins (p=0.003). Median p:c ratio for singletons was 0.15, and for twins 0.2. Groups differed in maternal age (mean 31.3 vs 35.3 years, p=0.0003) and gestational age at sample collection (35.6 vs 34.8 weeks, p=0.001), and were similar in weight, BMI, race, and smoking status. Using multiple logistic regression to control for the confounders of maternal age and gestational age yielded an adjusted OR for p:c ratio greater than 0.19 of 4.23 (1.61, 11.06). 43 of the 104 patients used ART (2 singletons, 41 twins) (p<0.0001). Number of fetuses and ART are highly correlated (r=0.77, p<0.0001) ARR was not a predictor of high pr:cr ratio (p=0.2447). ART was not a significant confounder of the relationship between pr:cr ratio and number of fetuses (p=0.0777) and lost significance in the presence of the other confounders, gestational age and maternal age (p=0.1496). Among twins, 21 patients with ART and 8 without had a high pr:cr ratio (p=0.1565). ART was not a significant predictor of pr:cr ratio (p=0.1156).

Conclusion
Women with uncomplicated twin pregnancies have higher rates of elevated protein to creatinine ratios than do women with singletons, suggesting that normal protein excretion in this group is greater than that in singleton gestations.

Perineal Body Length and Associations with Perinatal Outcomes

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Objective: To investigate associations between perineal body length (PBL) and perinatal outcomes such as lacerations and mode of delivery.

Methods: Prospective study of women with singletons who received prenatal care and delivered at our institution. PBL was measured from the posterior vaginal fourchette to the center of the anus to the nearest 0.5cm in the third trimester. Subesquent perinatal outcomes were examined using univariate and multivariable analyses.

Results: PBLs in our study population ranged from 2 to 7cm, with a median of 4cm. PBLs ≤3cm (≤10%ile) were considered short. Comparing women with short perineums to those with PBLs >3cm, 51.7% and 53.2% (p=0.894), respectively, experienced a perineal laceration during vaginal delivery (VD). However, Asian women demonstrated an aOR of 4.28 (p=0.041) for experiencing any perineal laceration during vaginal delivery (VD) after adjusting for PBL, ethnicity, parity, birthweight, and operative VD. Interestingly, 72.5% (79/109) of women with PBLs >3cm achieved VD, while 90.6% (29/32) of women with short PBLs achieved the same (p=0.033), a difference which was more pronounced when stratified by nulliparity (see table). In multivariable analyses, women with short PBLs demonstrated an aOR of 3.84 (95%CI:1.1-14.6) for achieving VD, after adjusting for prior vaginal delivery, birthweight, gestational age, and maternal age.

PBL and mode of delivery.

	Vaginal delivery	Cesarean delivery	p-value
PBL ≤3cm	90.6%	9.4%	0.033
PBL >3cm	72.5%	27.5%	0.033
PBL ≤3cm among nullips	100.0%	0.0%	0.016
PBL >3cm among nullips	70.2%	29.8%	0.016

Conclusion: Differences in perineal laceration rates were not observed between women with short PBLs and those with PBLs >3cm; however, women with short PBLs were found to have higher rates of achieving vaginal delivery, particularly among nulliparas. Further investigation will be valuable to determine factors contributing to this finding.

Twin Chorionicity and Association with Preeclampsia

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Objective: To determine whether monochorionic (MC) and dichorionic (DC) twins exhibit different rates of preeclampsia and severity of disease.

Methods: Retrospective cohort study of women with twin gestations from 2001-07. The primary outcome was preeclampsia with severity of disease as the secondary outcome. Women with MC pregnancies were compared to those with DC pregnancies to determine baseline rates of preeclampsia among these twin gestations, as well as to examine disease severity. The chi square test was used to compare proportions, with stratification by factors such as parity and maternal age. Multivariate analyses controlled for potential confounders.

Results: Of the 203 MC twins, 22 (10.8%) were associated with any preeclampsia, 10 (4.9%) with mild preeclampsia, and 12 (5.9%) with severe preeclampsia (see table). Of the 496 DC twins, 108 (21.8%) were associated with any preeclampsia, 70 (14.1%) with mild preeclampsia, and 38 (7.7%) with severe preeclampsia. DC twins were at increased risk of developing any preeclampsia (aOR 1.51, 95% CI 0.90-2.52) after controlling for parity, gestational age, maternal age, and assisted reproductive technology. However, among those with any preeclampsia diagnosis, MC twins exhibited higher odds of severe disease (aOR 1.60, 95% CI 0.57-4.53).

Preeclampsia and severity of disease by twin chorionicity. (* $p<0.05$)

	Any preeclampsia	Mild preeclampsia	Severe preeclampsia
MC	10.8% *	4.9% *	5.9%
DC	21.8% *	14.1% *	7.7%
All MC with preeclampsia diagnosis	--	45.5%	54.6%
All DC with preeclampsia diagnosis	--	64.8%	35.2%

Conclusion: While women with MC pregnancies were at lower risk of developing any preeclampsia compared to DC pregnancies, they demonstrated higher rates of severe disease. These findings yield insight into the pathophysiology of preeclampsia, and enable practitioners to better understand and counsel patients about this disease.

Title: Neonatal Bacteremia and Retinopathy of Prematurity: The ELGAN Study.

Authors: *Kristi Washburn Tolsma, MD[1], Elizabeth Allred, MS[2], Minghua Chen, MD, MPH[1], Jay Duker, MD[3], Alan Leviton, MD[2] and Olaf Dammann, MD, MS[1,2,4]. [1]Pediatrics Newborn Medicine, Floating Hospital for Children at Tufts Medical Center, Boston, MA, United States; [2]Neurology, Children's Hospital Boston, Boston, MA, United States; [3]Ophthamology, Floating Hospital for Children at Tufts Medical Center, Boston, MA, United States and [4]Perinatal Neuroepidemiology Unit, Hannover Medical School, Hannover, Germany.

Background: Retinopathy of prematurity (ROP) is a potentially disabling disorder of the developing retina and is an important and potentially preventable cause of blindness in childhood.

Objective: To test the hypothesis that preterm infants who have neonatal sepsis are at increased risk for more severe retinopathy of prematurity (ROP).

Design/Methods: We included 1059 infants born before the 28th week of gestation who were screened for ROP. We defined early definite bacteremia as a positive bacterial blood culture in the first week of life and late definite bacteremia as a positive bacterial blood culture in week 2, 3 or 4. Bacteremia was presumed if antibiotics were given for more than 72 hours despite negative blood cultures. Definite tracheal infection was defined as a positive tracheal culture for known pathogens. Time oriented risk modeling was used to adjust for confounders in order of their chronologic occurrence.

Results: Stage 3-5 ROP occurred in 39% of infants with documented early bacteremia, 33% with presumed early bacteremia and in 25 % with no early bacteremia. The respective numbers for late bacteremia are 34%, 42% and 22%. In multivariable models adjusting for gestational age and other potential confounders, both definite tracheal infection (OR 1.7, 1.1-2.6) and presumed or definite late bacteremia (OR 1.5, 1.2-2.1) were associated with Stage 3-5 ROP.

Conclusions: Infants exposed to late neonatal bacteremia appear to be at increased risk for Stage 3-5 ROP. Time-Oriented models including specific variables of infection are warranted to further delineate risk patterns.

Factors Predicting Successful Discontinuation of Supplemental Oxygen (supO$_2$) in VLBW Infants Approaching NICU Discharge JM Trzaski*[1,2,3], JI Hagadorn[2,3], C Wittenzellner[1], J Schwenn[1] and N Hussain[1,3]. [1]Neonatology, Univ of CT Health Ctr, Farmington, CT; [2]Neonatology, Connecticut Children's Med Ctr, Hartford, CT and [3]Pediatrics, Univ of CT Sch of Med, Farmington, CT.

Background: Stress oximetry testing (SOT) is a standardized set of graded clinical maneuvers assessing readiness for discontinuing supO$_2$ in VLBW infants. **Objective:** To use SOT to identify clinical factors associated with readiness for discontinuing supO$_2$ in VLBW infants approaching NICU discharge. **Design/Methods:** From 2004-2009 SOTs were performed weekly on VLBW infants ≥34 wks PMA on supO$_2$ via nasal cannula (NC), with clinical and SOT outcome data collected prospectively. A failed SOT was defined prospectively as SpO$_2$ <90% for >1 minute, increased WOB (nasal flaring, shoulder heaving or head bobbing) above baseline, or WOB preventing PO intake of 120 ml/kg/day. We randomly divided SOTs into cohorts for model derivation (2/3) and testing (1/3), and performed comparative and hierarchical logistic regression analyses, adjusting for clustering of multiple tests within infants. Model discrimination and calibration were assessed. **Results:** 233 infants on NC supO$_2$ (BWt 901±245 g, GA 26.9±1.8 wks) had 988 SOTs at 38.6±2.6 wks PMA. SOTs were divided into derivation (n=676) and test (n=312) cohorts. In regression analysis, increasing weight at time of SOT was associated with increased adjusted odds of pass. History of PDA ligation and increasing capillary pCO$_2$, N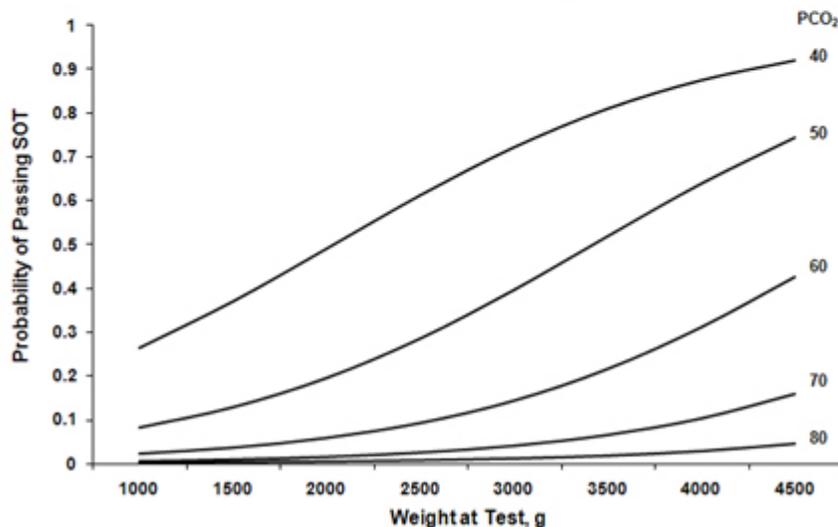C flow rate, and pulmonary acuity score (PAS) decreased odds of SOT pass (Table). ROC curve area was 0.82 for both derivation and test cohorts. The model performed well within pCO$_2$ and PAS subgroups. Predicted probability of passing SOT by weight and pCO$_2$ is shown for an example hypothetical group of infants on NC supO$_2$ (Figure). **Conclusions:** Weight on test day, pCO$_2$, NC flow rate, pulmonary acuity score, and history of PDA ligation predict SOT outcome. These factors may be used to predict which infants are ready to have supO$_2$ discontinued and will continue to grow and thrive. This model may be useful for management and discharge planning for VLBW infants on NC supO$_2$. Independent prospective validation of this predictive model is warranted.

TABLE: Factors associated with passing stress oximetry test

	Odds Ratio[1]	95% CI	p-value
Weight, day of test (per 100 g)	1.10	1.06, 1.15	<0.001
pCO$_2$, mm Hg	0.87	0.84, 0.91	<0.001
History of PDA ligation	0.38	0.22, 0.65	0.001
Cannula flow, l/min	0.20	0.11, 0.38	<0.001
Pulmonary acuity score[2]	0.11	0.02, 0.46	0.003

[1]OR > 1 indicates increased odds of passing stress oximetry test.
[2]Components of Pulmonary Acuity Score (Madan et al 2005): effective FiO2, systemic steroids, inhaled steroids, diuretics, caffeine

FIGURE: Predicted probability of passing SOT by weight and pCO$_2$

Example predicted probability curves for infants on NC 0.25 L/min, no history of PDA ligation, PAS 25th percentile. Curves will vary with cannula flow, ligation history, pulmonary acuity score.

Interaction of Cerclage and Cervical Length: An Analysis of Median Cervical Lengths in Singleton and Multifetal Pregnancies

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Background:
Despite evidence of benefit in singleton pregnancies, recent work suggests that cerclage placement may actually be detrimental in multifetal pregnancies. We hypothesized that there is a significant interaction between cervical length and twin gestations pregnancies treated with transvaginal cerclage.

Methods:
This is a retrospective review of 24 week transvaginal cervical length measurements at our institution over the past 10 years. Included are all patients who underwent cerclage placement at <16 weeks gestation (prior to cervical length screening) for historical indications. A bivariate analysis was performed using a rank-sum test to compare singleton and twin pregnancies. A quantile regression was used to estimate the median of our target variables.

Results:
The maternal age, gravidity and parity in our sample of 503 singleton and 99 twin gestations were not significantly different. The median cervical length at 24 weeks was 2.6cm and 1.8cm for singleton and twins respectively (p<0.001). The median gestational age at delivery was 38 and 34 weeks respectively (p<0.001). In a multivariate quantile regression of the median gestational age at delivery, there is a significant interaction between cervical length and twins (Table 1).

Table 1

Variable	Coef	95%CI	p-value
Twin	-5.2	(-6.0, -4.4)	<0.0001
Cvx length (cm)	-2.2	(-2.0, -2.4)	<0.0001
Twin-Cvx length interaction	-0.8	(-0.45, -1.2)	<0.0001
Funneling	-1.3	(-0.89, -1.7)	<0.0001

Conclusions:
The interaction between cervical length and twin gestation suggests that the risk of early delivery associated with twin gestations with cerclage is more than a function of cervical length alone. Compared with singleton gestations, twins manifest a unique risk in the setting of short cervix and cerclage.

Need for patent ductus arteriosus (PDA) ligation is increased in infants exposed to prenatal indomethacin

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Background: Indomethacin, a cyclooxygenase (COX) inhibitor, is used to suppress preterm labor as well as to treat clinically significant PDA. Thus premature infants may get exposed to this medication consecutively, once in utero and then after birth. There is some evidence that prenatal indomethacin exposure worsens the severity of PDA. In this study we investigated whether prenatal exposure to indomethacin alters effectiveness of COX inhibitors (indomethacin or ibuprofen) in causing PDA closure. We used need for surgical PDA ligation as an indicator of failure of medical therapy for PDA.

Objective: To study the effect of prenatal indomethacin on incidence of PDA ligation in high-risk newborn infants.

Design/Methods: We selected all infants born at < 32 weeks and with hemodynamically significant PDA from among all admissions to the University of Connecticut Health Center NICU from Jan 1990 to Jun 2009. Previously collected data from a neonatal database was used to compare putative risk factors.

Results: Our study group consisted of 966 infants who were born at ≤32 weeks and had hemodynamically significant PDA. Of these 11% (106) required surgical ligation due to lack of response to indomethacin or ibuprofen. Comparison of infants with and without surgical ligation showed statistically significant relationships with GA (mean: 25 wk vs. 27 wk; $p < 0.0001$), BW (mean: 825 g vs. 1017 g; $p < 0.0001$), sex (F>M p=0.01). Eighty two infants were exposed to indomethacin prenatally and there was no difference in the incidence of PDA between this group and the rest of the study group. However, the incidence of PDA ligation in infants exposed to prenatal indomethacin was 16.4% (20/82) as compared to 9.7% (86/884) in infants not exposed to prenatal indomethacin.

Prenatal indomethacin versus PDA ligation			
	No prenatal indomethacin	Yes prenatal indomethacin	Totals
No PDA ligation	798	62	860
Yes PDA ligation	86	20	106
Totals	884	82	966

This difference was statistically significant. On multiple logistic regression analysis, after adjusting for statistically significant variables (GA, BW, and sex), significant relationship remained between prenatal indomethacin exposure and need for surgical ligation (OR 2.573, 95% CI 1.455, 4.55, p=0.012).

Conclusions: Prenatal indomethacin exposure is associated with decreased effectiveness of postnatal medical therapy for PDA and may therefore increase the risk of needing PDA ligation in premature infants.

Evaluation Of A Tablet PC-Based Survey And Educational Tool For Prenatal Diagnosis And Aneuploidy Screening

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Objective: To evaluate the effectiveness of a tablet PC-based tool designed to collect medical history via a self-administered questionnaire and present options for aneuploidy screening.

Methods: We surveyed patients about their experience using tablet PC-based software. The survey was conducted at a single OB/GYN practice and a single maternal-fetal medicine referral office. IRB approval was obtained.

Results: We obtained data on 21 patients who used the tablet PC-based software before being seen for a prenatal visit or prenatal diagnosis visit. A majority of respondents were Caucasian, college-educated, and married. Among respondents, 96 percent found the software easy to use, and 59 percent thought it saved time. 91 percent found the information about aneuploidy screening to be informative, but 14 percent reported that they would have preferred to be questioned directly by a staff member.

Conclusions: Clinical decision-support software may facilitate education and data-collection in prenatal care.

Do Biopsy Forceps or the Aspiration Cannula Produce a Better Chorionic Villus Sampling Specimen?

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Objective: To compare the CVS biopsy quality resulting from CVS done using biopsy forceps and the aspiration cannula.

Methods: Nine women diagnosed with pregnancies at high-risk for aneuploidy (n=3) or with missed abortion (n=6) undergoing CVS had samples taken with both biopsy forceps and the aspiration cannula. A cytopathologist blinded to the origin of the sample evaluated each specimen using standard criteria.

Results: Chorionic villi were obtained successfully for each patient using the biopsy forceps, but in only five with the cannula (including the three ongoing pregnancies). The mean sample size obtained using the biopsy forceps was 12.2mg and using the aspiration cannula was 8.6mg. In two missed abortions (in which chorionic villi were not retrieved by aspiration), a karyotype could not be provided and 45,X was diagnosed by QF-PCR . Trisomy was present in six of the seven remaining cases (three 21, one 22, one 20, and one 16). In the blinded sample quality assessment, samples obtained using biopsy forceps were cleaner, had less mucus and fewer clots, but had more decidual tissue than samples obtained using the cannula.

Conclusion: CVS biopsy forceps produces a better specimen for evaluation than the aspiration cannula.

Physician Administration of Prophylactic Indomethacin in Premature Neonates

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Background: Administration of prophylactic indomethacin to premature neonates to prevent or limit the sequelae associated with patent ductus arteriosus (PDA) and intraventricular hemorrhage (IVH) remains controversial among practicing neonatologists.

Objective: To examine self-reported physician preferences and clinical outcome perceptions regarding the use of prophylactic indomethacin in preterm neonates.

Methods: An anonymous email questionnaire survey was distributed to 1,652 practicing U.S. neonatologists. The overall use of prophylactic indomethacin, and clinician perceptions of risks, benefits, and short and long term clinical outcomes were assessed.

Results: Response rate was 25%. Only 43% of responders used indomethacin in practice. Of those, most believed that it reduced the incidence of grade III-IV IVH (93.6%), symptomatic PDA (84%) and need for surgical PDA ligation (81%) in infants <28 weeks' gestation and < 1000 gm. Nonusers believed the drug was associated with short term harm (64%), specifically NEC (61%), and felt the current literature did not support long-term benefits (91%). Of all responders, 57% felt the benefits of prophylactic indomethacin did not outweigh the risks.

Conclusions: Great variability regarding the use of prophylactic indomethacin exists among practicing U.S. neonatologists. Despite this discrepancy in physician preference, both users and nonusers cite a paucity of current clinical evidence for long-term benefits associated with the use of prophylactic indomethacin in the premature neonatal population. These findings suggest the need for large randomized clinical trials to delineate the risks and benefits associated with the use of prophylactic indomethacin.

A Prospective Study of 2nd trimester Leptin Level and Failed First Stage of Labor

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Objective
Increasing evidence suggests leptin affects myometrial contractility. We therefore examined the extent to which second trimester leptin levels were associated with cesarean delivery for failed first stage of labor independently of maternal body mass index (BMI).

Methods
We included 1512 participants in the cohort study Project Viva who had second trimester plasma leptin levels and pregnancy outcomes. We abstracted the indication for cesarean delivery from operative reports and defined failed first stage of labor as cesarean delivery for arrest of dilation, "failure to progress" or failed induction of labor. We then performed multivariate logistic regression to assess the odds for failed first stage according to leptin level, before and after controlling for maternal pre-pregnancy BMI and other covariates, and stratified by parity.

Results
325 (21.5%) participants were delivered by cesarean section (CS), of whom 87 (5.8%) had a CS for failed first stage of labor. Mean $\pm$ SD leptin level was 23.3 $\pm$ 14.3 ng/mL, and leptin was strongly correlated with maternal pre-pregnancy BMI (r=0.61, p<0.0001). Mean leptin levels were higher in women with CS for failed first stage than those without (26.5 vs. 23.2, p=0.03). Among nulliparous women, after adjusting for maternal age, race/ethnicity, education, smoking, household income and birthweight for gestational age z-score, but before adjusting for maternal BMI, leptin level predicted CS for failed first stage of labor (OR 1.14 per 10 ng/mL, 95%CI 0.96, 1.37.) However, after further adjustment for maternal BMI, the OR was 0.99 (95% CI 0.80, 1.24.) Among multiparous women, the corresponding odds ratios were 1.01 (95% CI 0.77, 1.32) before BMI adjustment and 0.78 (95% CI 0.54, 1.13) afterwards.

Conclusion
Although murine and human tissue culture evidence suggests that leptin inhibits myometrial contraction in a dose-related fashion, we found that the relationship between mid-pregnancy serum leptin level and cesarean delivery for failed first stage of labor was fully explained by their associations with maternal BMI.
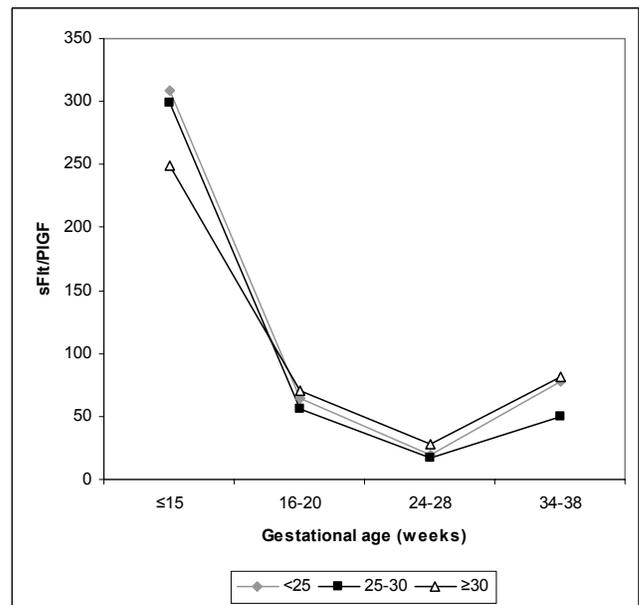
A Prospective Study of Obesity and Serum Angiogenic Markers in Normal Pregnancies

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Objective: As increased BMI is a risk factor for preeclampsia (PE), we sought to assess whether maternal obesity influences serum angiogenic markers associated with PE in normal pregnancies. **Study Design:** 339 women with singleton gestations were enrolled at Brigham and Women's Hospital as part of a multi-center prospective cohort designed to evaluate the utility of serum angiogenic markers for the early prediction of PE. Blood and urine samples were collected in each trimester, near term and at delivery, and demographic and pregnancy outcomes were abstracted. Blood samples were analyzed for soluble Fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF). 7 women who developed PE were excluded from the analysis. We performed linear regression to assess the relationship between first trimester body mass index (BMI) and sFlt-1, and longitudinal regression to assess the effect of BMI on sFlt-1:PlGF ratio over gestation. **Results:** 49 women were obese (BMI > 30), 84 were overweight (BMI 25-30) and 199 were normal weight (BMI<25) in the first trimester. Obese women were less likely to be Caucasian than normal weight women (p<0.0001). Obese women had lower mean first trimester sFlt-1 levels than women with a BMI in the normal range (p=0.00179, Table 1). When compared to normal weight women in a multivariate model adjusting for diabetes and parity, women with a BMI > 30 had a significantly different trend in sFlt:PlGF ratio over the length of gestation than normal weight women (p=0.0033; Figure 1.) **Conclusion:** Maternal BMI affects sFlt-1 and sFlt-1:PlGF ratio in pregnancies not complicated by PE. Further study is needed to fully characterize the relationship between BMI and serum angiogenic markers implicated in PE. Algorithms that incorporate these markers for the prediction of PE may need to consider the impact of maternal BMI.

Figure 1: Mean sFlt-1/PlGF ratios by baseline BMI, longitudinal regression

Table 1: Mean (SD) sFlt-1 at each visit, by BMI category, F test for linear regression

	Baseline BMI						
	<25 (N=199)		25-30 (N=84)		≥30 (N=49)		p-value
sFlt ng/mL, mean (sd)							
≤15 weeks gestation	6166.28	(3969.38)	6264.93	(3375.62)	4591.42	(2496.54)	0.0179
16-20	7719.89	(6243.58)	6524.60	(3282.47)	6445.61	(3713.56)	0.1364
24-28	7484.25	(5726.65)	6504.07	(4429.04)	6585.87	(3719.09)	0.2795
34-38	12157.20	(9142.15)	10301.41	(5949.63)	11008.29	(7563.55)	0.2231