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EFFECT OF STORAGE TIME IN THE *IN VIVO* FUNCTION OF A NEW EXOGENOUS PULMONARY SURFACTANT

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Background: The function of exogenous pulmonary surfactant depends of an adequate stability of its components, a highly complex mixture of lipids (mainly phospholipids) and two hydrophobic polypeptides, SP-B and SP-C. This composition could become instable with long time surfactant storage. The Butantan Institute (Brazil) produced a new porcine pulmonary surfactant preparation composed mainly of 76% of phosphatidylcholine (30–35% of the phosphatidylcholine is saturated dipalmitoyl phosphatidylcholine), 6–8% of phosphatidyllethanolamine, 6% of phosphatidylinositol+phosphatidylserine, and 4–6% of sphingomyelin, with 5.6% of two hydrophobic polypeptides, SP-B and SP-C. The objective of this study was to evaluate the Butantan exogenous surfactant function one year after its production, using the premature rabbit model.

Methods: 16 New Zealand White premature rabbits were delivered by c-section at 27 days gestation and randomized into two study groups according to the type of surfactant treatment: Butantan surfactant (n=8, animals treated with Butantan surfactant kept in refrigerator at 4o C for one year), and Curosurf (Farmalab-Chiesi) group (n=8; surfactant used 4 months after its production). Animals were ventilated with a preset tidal-volume of 8 ml/kg for 15 minutes, using a ventilator-pletismograph system with the following ventilator settings: respiratory rate (RR), 60 cycles/min; FiO2, 0.21; peak inspiratory pressure (PIP) needed to acquire the target tidal-volume (8 ml/kg); PEEP, 0 cmH2O; inspiratory and expiratory time, 0.5 seconds. Ventilatory pressure (VP=PIP-PEEP), tidal-volume (TV), and dynamic compliance (DC=TV/VP) were recorded each five minutes until sacrifice. Statistical analysis was performed by t-test. Significance level was set at 0.05.

Results:

Study group	Butantan	Survanta
Body weight (g)	30.0±2.6	29.9±3.0
Dynamic compliance (ml/kg.cmH2O)	0.569±0.083	0.0538±0.130
Ventilatory pressure (cmH2O)	14.5±2.1	15.7±3.4
Tidal volume (ml/kg)	8.1±0.2	8.1±0.1

Conclusion: The Butantan surfactant showed to be as effective as a newly synthesized Curosurf to treat respiratory distress syndrome in the premature rabbit model, one year after storage at 4°C in refrigerator.

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EARLY INCREASE OF NITRIC OXIDE IN A MODEL OF PERINATAL ASPHYXIA IN FETAL LAMBS

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Background: Endogenous nitric oxide (NO) production increases in perinatal asphyxia and inflammatory processes. Histological lesion (necrosis and/or apoptosis) secondary to hypoxic-ischemic (H-I) injury appears in several hours after the insult but biological markers appear earlier. In our previous studies (1,2) we have observed early changes in cerebral blood flow and in O2-uptake. Aim: To study the early changes of serum NO concentration in a model of perinatal asphyxia induced by prolonged umbilical cord clamping in premature lambs.

Methods:10 preterm lambs (80–90% GE) were randomly assigned to: Control group, after Cesarean section, lambs were managed on conventional mechanical ventilation for 3 hours (n=5); Asphyctic group, H-I injury was performed by partial cord clamping during 60 min and later, lambs were managed similar to the control group (n=5). NO was measured in serum samples by fluorometric assay at baseline (B), immediately after H-I injury and at the end of experiment (3h). Comparation were performed by one-factor ANOVA, $p<0.05$.

Results:: NO concentration results are summarised in table:

NO(μM)	B	H-I	3h
CONTROL	0.54±0.05	0.54±0.05	0.53±0.13
ASPHYTIC	0.62±0.18	0.87±0.24(*)	0.88±0.24(*)

(*)vs CONTROL group

Conclusion: In our model of perinatal asphyxia by partial occlusion of umbilical cord in premature fetal lambs, cellular injury can be assessed just at the end of H-I episode. This early change could be used to test the effects of early preventive strategies in the H-I injury. (1) Alvarez et al. Cerebral O2 uptake and blood flow of asphyxiated lambs on liquid ventilation and MgSO4. Pediatr Res 2001;50:278A. (2) Alvarez et al. Regional cerebral blood flow and O2-uptake changes in preterm lams with hypoxic-ischemic injury rescued with MgSO4. Pediatr Res 2002;51:454A. Supported by grants: FIS 01/0110–01,-02 and RESPIRA net of RITC, FIS C03/11.

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CHANGES IN SURVIVAL AND NEURODEVELOPMENTAL OUTCOME IN 22 TO 25 WEEK GESTATION INFANTS OVER A 20 YEAR PERIOD

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Background: Advances in neonatal care have resulted in the survival of increasing numbers of extremely preterm infants. There have been concerns that the improved survival of infants born at the limit of viability has resulted in infants surviving with significant disability who would have previously died. The aim of this study was to examine the change in survival over a 20 year period of infants born at less than 26 weeks gestation at a single tertiary referral centre and to correlate this with neurodevelopmental outcome at age 1 year.

Methods: All infants without major congenital abnormalities born at less than 26 weeks gestation and admitted to University College London Hospitals neonatal unit between 1981 and 2000 were prospectively recruited for long term follow-up. At one year of age corrected the infants were recalled. Each child underwent a Griffiths Developmental Assessment and an Amiel-Tison neurological examination. Neurological status was then classified as normal, impairment without disability or impairment with disability. The data were divided into 5 year blocks for analysis.

Results: A total of 353 infants fulfilled the recruitment criteria and were enrolled into the study. Of these 190 died and 159 were recalled at one year of age. Data on 4 infants was not available. Survival to one year during the time periods 1981–1985, 1986–1990, 1991–1995 and 1996–2000 was 32%, 34%, 46% and 71% respectively. The increased survival seen during the 1996–2000 period was statistically significant using Chi Squared analysis ($p = 0.002$). Statistical analysis of the Griffiths results was limited by modification of the test which resulted in restandardisation in 1996. This led to an overall reduction in the mean developmental quotient of 11 points in the normal population. For the infants in our study the mean total developmental quotient (DQ) during each of the time periods fell within the normal range. Values are shown in table 1. Analysis of the sub-scales revealed similar findings. The incidence of neurodisability was unchanged over the time periods ($p=0.18$). See table 1.

Conclusion: Our data show that despite a significant increase in the survival of infants born at less than 26 weeks gestation over a 20 year period there has been no significant increase in the incidence of adverse neurodevelopmental outcome in survivors.

Table 1

Year	Griffiths Total DQ(SD)	Normal neuro-development No(%)	Impairment without disability No(%)	Impairment plus disability No (%)
1981–1985	105.0(16.6)	12(46%)	9(35%)	5(19%)
1986–1990	106.1(17.6)	19(55%)	7(21%)	8(24%)
1991–1995	103.9(19.2)	8(29%)	9(32%)	11(39%)
1996–2000	94.9(16.2)	30(57%)	9(17%)	14(26%)

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OVEREXPRESSION OF *WNT7A* IN TRANSGENIC MOUSE NEURAL STEM CELLS INCREASE *VANGL2* EXPRESSION AND IMPAIR NEURULATION BY DISTURBING ACTIN MICROFILAMENT FORMATION

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Background: The Wnt gene family encode secreted glucoproteins that function in intercellular signalling. They play essential roles in shaping the nervous system during embryonic development. The Wnts signal via at least 3 different pathways. These include the b-catenin, the planar cell polarity (PCP) and the calcium pathways. Wnt signalling can inhibit the cells continous degradation of b-catenin degradation, thus allowing â-catenin to translocate to the nucleus were it in association with transcription factors cause target gene transcription. â-catenin is also associated with adherens junctions, were it acts as an anchor for cadherins involved in cell-cell contacts, and for actin microfibers of the cytoskeleton. The planar cell polarity and the calcium pathways are believed to be important regulators of gastrulation and neurulation in vertebrates.

Methods: Fertilized mouse oocytes were injected with a gene construct which utilize the nestin gene to direct expression of Wnt7a to neural stem cells. The treated oocytes were implanted into pseudopregnant females and allowed to develop for 7,5–14,5 days. The embryos were then genotyped by PCR, cryosectioned and studied by standard histochemichal methods.

Results: Embryos overexpressing Wnt7a displayed aberrant morphologies that seemed to be related to a defective neurulation. Surprisingly, the transgenic embryos partly phenocopied mouse embryos that had been let to develop in the presence of cytochalasin, a substance that disrupts actin microfilaments. â-catenin immunofluorescence was reduced over adherence junctions of transgenic embryos. Double labelling with phalloidin revealed an overlapping reduction in actin microfilaments. Expression of a gene essential for the PCP pathway, *Vangl2*, was upregulated in the transgenic embryos.

Conclusion: The increased *Vangl2* expression indicate that Wnt7a can activate the PCP pathway in mammals. This in itself can lead to an impaired neurulation by affecting cell migration. However, part of the phenotype observed in the transgenics was likely due to the reduced levels of â-catenin and actin microfilaments at the adherens junctions. This could either be an effect of the PCP and the â-catenin pathway counteracting each other, or since the PCP gene Scribble binds to adherens junctions in *Drosophila*, by a direct effect of PCP signaling on adherens junctions. This demonstrates that a Wnt ligand can be bioactive also by affecting â-catenins function as a part of the cytoskeleton.

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EFFECT OF BLOOD SAMPLING ON CEREBRAL OXYGENATION IN VLBW INFANTS: HEEL LANCE VERSUS VENEPUNCTURE

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Background: We have shown before that blood sampling from umbilical artery catheters induces a decrease in cerebral oxygenation and cerebral blood volume (CBV) in VLBW infants. The aim of the present study was to assess, if heel lance and venepuncture effect cerebral oxygenation as well.

Methods: Seventeen preterm infants (birth weight 355 – 1280 g, median 790 g; gestational age 24 - 32 weeks, median 27 weeks) were studied. In 11 cases, heel lance and venepuncture blood sampling were analyzed, in 3 heel lance, and in 3 venepuncture only. Using near infrared spectroscopy changes in oxygenated cerebral hemoglobin (O2Hb) and deoxygenated hemoglobin were measured and changes in cerebral oxygenation (HbD) and CBV were calculated. Arterial oxygen saturation, heart rate, blood pressure and tcPCO2 were registered simultaneously.

Results: Blood sampling induced a decrease in cerebral O2Hb (heel lance: -3.1 micromol/L; $p = 0.004$; venepuncture: -1.7 micromol/L; $p = 0.06$) and HbD (heel lance: -4.4 micromol/L; $p = 0.008$; venepuncture: -3.3 micromol/L; $p = 0.02$) compared to baseline levels which was more pronounced after heel lance. CBV decreased significantly after heel lance blood sampling only. Simultaneously, arterial oxygen saturation as measured by pulse oximetry dropped significantly (from 92.5% to 88.7% during heel lance; $p = 0.03$; from 92.3% to 89.6% during venepuncture; $p = 0.05$). Small but significant changes of heart rate and blood pressure were observed.

Conclusion: Heel lance and venepuncture for blood sampling induce a decrease in cerebral oxygenation. The decrease is more pronounced and lasting in heel lance compared to venepuncture and it is even of greater magnitude than the decrease during blood sampling from umbilical artery catheters. We speculate that the decrease in cerebral oxygenation during blood sampling from umbilical artery catheters is caused by acute volume loss, whereas the decrease during heel lance and venepuncture might be caused by a stress induced decrease in arterial oxygen saturation.

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NEONATAL PULSE OXIMETRY SCREENING FOR CRITICAL CONGENITAL HEART DISEASE

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Background/aims: Critical congenital cardiovascular malformations (CCVMs) have been defined as lesions likely requiring surgical correction during the first month of life. CCVMs are relatively common, with a prevalence of 5 -10 in every 1000 live births. Routine physical examination is unable to detect > 50% of CCVMs infants. Our aim was to verify the recent report by Koppel et al. (Pediatrics 2003;111:451–5), suggesting that a pulse oximetry screening, based on a single determination of postductal saturation (SpO2), is a noninvasive and highly specific test for early detection of CCVMs.

Methods: Oximetry was performed on 4197 asymptomatic newborns discharged from nursery at median age of 72 hours during the 2000 May 1 - 2004 March 31 period. Infants symptomatic before screening (heart murmur, severe cyanosis) and those with suspected lesions detected by fetal echocardiography were excluded. Cardiac ultrasound was performed on all infants with SpO2≤95% at > 24 hours (positive screens).

Results: Two cases of CCVMs were detected (intracardiac total anomalous pulmonary venous return with post-ductal SpO2 88% and ductal-dependent aortic coarctation with SpO2 85%). Both infants received surgical correction before the first month of life. There were no false-positive screens. One infant with negative screen was readmitted at 26 days of age for a non ductal-dependent aortic coarctation (false negative screen). The mean sensitivity value of the SpO2 was 66.7%, with 100% specificity, 100% positive predictive, and 100% negative predictive value. During the study period, the prevalence of critical CCVMs among the screened population was 1 in 1399.

Conclusion: Our findings confirm that neonatal pulse oximetry screening is a satisfactorily accurate, simple, noninvasive and inexpensive test for early detection of CCVMs which could be applied extensively especially to asymptomatic newborns in well-infant nurseries before discharge.