Brain inflammation and injury resulting from chorioamnionitis is exacerbated by resuscitation in preterm lambs

G.R. Polglase,[1] S.K. Barton,[1] S.B. Hooper,[1] S.L. Miller,[1] T.J.M. Moss,[1] F. Wong,[1] K.C. Crossley,[1] A.W. Gill,[2] M. Kluckow[3] and M. Tolcos,[1] [1]The Ritchie Centre, Monash Institute of Medical Research, Monash University, Clayton, VIC 3168, Australia, [2]Centre for Neonatal Research and Education, School of Women's and Infants' Health, The University of Western Australia, Subiaco, WA 6009, Australia and [3]Department of Neonatology, Royal North Shore Hospital, University of Sydney, NSW 2065, Australia. (Introduced by Alistair Gunn)

Background: Premature infants are often born with brain injury as a consequence of being exposed to inflammation *in utero* (chorioamnionitis). In addition, these preterm infants often require mechanical ventilation at delivery, a regime that in itself, is associated with brain injury. We have previously identified that the initiation of mechanical ventilation using high tidal volumes (V_T; injurious ventilation) imitates that inadvertently received by preterm infants in the delivery room (Schmölzer *et al.*, 2010), adversely affected cerebral haemodynamics and caused brain inflammation; this then increased the incidence and severity of white matter injury. However, a gentle ventilation strategy (using a lower V_T) reduced the incidence and severity of brain injury (Polglase *et al.*, 2012). The current study aims to determine the effects of injurious and gentle ventilation strategies on the preterm brain following intrauterine inflammation. We hypothesize that intrauterine inflammation will exacerbate the adverse effects of high V_T resuscitation and that a gentle ventilation strategy will be less damaging to the immature brain.

Methods: Ultrasound guided intra-amnioitc injection of either lipopolysaccharide (LPS) or saline (SAL) was administered to preterm lambs 2 d prior to delivery and resuscitation. Lambs were delivered *via* caesarean section under inhalational anaesthesia (Isoflurane), were surgically instrumented for measurement of systemic and cerebral blood pressures and flows and were delivered, dried and warmed. Ventilation was initiated (Drager Babylog 8000+) using either an injurious high V_T strategy (V_T of 12 mL/kg for 15 min followed by V_T 7 mL/kg for 75 min; LPS_{INJ}) or a gentle strategy (prophylactic surfactant (480 mg Curosurf, Chiesi), a 20 s sustained inflation followed by V_T 7 mL/kg for 90 min; LPS_{GENT}). Cardiovascular variables were determined using echocardiography and cerebral regional oxygenation measured using near infrared spectroscopy. At the end of ventilation, lambs were euthanased (Pentobarbitone; Lethabarb 150 mg/kg i.v.) and brains were collected for histological, immunohistochemical and molecular assessment of injury, inflammation, hypoxia, vascular leakage and angiogenesis. Unventilated lambs were used as controls; LPS_{UVC} and saline (SAL_{UVC}). 2-way or 1-way repeated measures ANOVA as appropriate, determined significance.

Results: Oxygenation, lung compliance and cerebral tissue oxygenation index were lower in LPS_{INJ} lambs compared to LPS_{GENT} lambs. Exposure to chorioamnionitis significantly increased brain inflammation (inflammatory cell recruitment; $P<0.05$), blood brain barrier permeability $P<0.05$, decreased cerebral vessel density $P=0.076$, and tended to decrease mRNA expression of VEGFR-2 ($P=0.067$). Ventilation after chorioamnionitis increased histological indices of brain injury as well as pro-inflammatory cytokine mRNA expression of IL-8 $P=0.002$, and further increased blood brain barrier permeability ($P<0.05$), oxidative damage, cell death ($P=0.003$) and astrogliosis ($P<0.001$). The increase in brain inflammation and injury after chorioamnionitis occurred irrespective of the ventilation strategy used.

Conclusion: Acute chorioamnionitis causes brain inflammation and injury, decreases vascular density and markers of angiogenesis. Ventilation increased brain inflammation and injury irrespective of the whether injurious or gentle strategies were used.

Polglase GR, Miller SL, Barton SK, Baburamani AA, Wong FY, Aridas JD, Gill AW, Moss TJ, Tolcos M, Kluckow M, Hooper SB. (2012) Initiation of resuscitation with high tidal volumes causes cerebral hemodynamic disturbance, brain inflammation and injury in preterm lambs. *PLoS ONE* **7:** e39535.

Schmölzer GM, Kamlin OC, O'Donnell CP, Dawson JA, Morley CJ, Davis PG. (2010) Assessment of tidal volume and gas leak during mask ventilation of preterm infants in the delivery room. *Archives of Diseases in Childhood. Fetal and Neonatal Edition* **95:** F393-397.