Gestational age at time of *in utero* lipopolysaccharide exposure influences the severity of inflammation-induced diaphragm dysfunction in lambs

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Intra-uterine inflammation, commonly manifesting as chorioamnionitis, is associated with up to 70% of extreme preterm births and may contribute to adverse respiratory outcomes. The resilience of infants to developing respiratory failure after birth may be critically influenced by the integrity of the diaphragm. Our previous studies suggest that the timing of *in utero* lipopolysaccharide (LPS) exposure critically influences the severity and molecular mechanisms responsible for inflammation-induced diaphragm weakness in preterm lambs. However, it is unclear whether the diaphragm of term lambs is also vulnerable to *in utero* LPS exposure. Given the structural and functional immaturity of the preterm diaphragm, we hypothesized that preterm lambs are more susceptible to inflammation induced diaphragm dysfunction than their term counterparts.

This study was approved by the Animal Ethics Committee of The University of Western Australia and animal experiments were conducted according to NHMRC guidelines for the use of animals in experimental research. Pregnant Merino ewes received ultrasound guided intramaniotic (IA) injection of LPS (10 mg in 2 mL saline: *Escherichia coli* 055:B5, Sigma-Aldrich, St Louis, MO) or an equal volume of saline (Sal) at 2 d or 7 d prior to delivery at 121 d (preterm) or 145 d (term) gestational age (GA). There were no significant differences between 2 d and 7 d LPS within the same GA, therefore data were pooled to generate single LPS group for each GA. Lambs were delivered *via* caesarean section and immediately euthanised by pentobarbitone overdose (150 mg/kg IV). Longitudinal strips of muscle fibres were dissected from the right hemi-diaphragm and mounted in an *in vitro* muscle test system (model 1205, Aurora Scientific In., Canada) for assessment of contractile function including maximum tetanic force (P_0) and maximum twitch force (P_t) and susceptibility to fatigue. Muscle fibre myosin heavy chain (MHC) isoform composition, inflammatory cytokine response and markers of oxidative stress and proteolysis were evaluated using immunofluorescence staining, ELISA and biochemical assays.

In utero LPS exposure resulted in a similar inflammatory response (increased plasma IL-6 concentration) in both term and preterm lambs, however the extent of diaphragm contractile dysfunction varied with GA. In term lambs, P_0 after LPS exposure (16.0 ± 3.2 N.cm^2, n = 12; mean ± SD) was ~20 % lower than saline treated control lambs (20.1 ± 3.7 N.cm^2, n = 9; P<0.05). In preterm lambs, however, P_0 was ~30 % lower than control lambs (LPS: 10.9 ± 2.2 N.cm^2; n = 12; Sal: 15.6 ± 3.6 N.cm^2; n = 8; P<0.05). Whereas P_t was not affected by LPS exposure in term lambs (LPS: 8.1 ± 2. N.cm^2; n = 12; Sal: 9.5 ± 2.2 N.cm^2; n = 6; p > 0.05), it was ~30 % lower in preterm lambs exposed to LPS compared to their controls (LPS: 5.6 ± 1.5 N.cm^2; n = 12; Sal: 8.2 ± 2.1 N.cm^2; n = 7; P<0.05). In term lambs, fatigue resistance was significantly greater (by ~45 %) after LPS exposure relative to controls, and was accompanied by a significant increase in the proportion of slow (MHCs positive) fibres (LPS: 27.8 ± 6.6 %; Sal: 21.5 ± 6.0 %; P<0.05) and a decrease in fast (MHCf positive) fibres (LPS: 66.7 ± 5.0 %; Sal: 72.3 ± 5.3 %; P<0.05). No changes in fatigue resistance or myofibre composition were observed after LPS exposure in preterm lambs. Furthermore, LPS exposure had no significant effect on markers of oxidative stress or proteolysis in either term or preterm lambs.

These findings indicate that preterm lambs are more vulnerable to diaphragm dysfunction induced by *in utero* exposure to inflammation compared to term lambs. Although the term diaphragm was also susceptible to inflammation induced diaphragm weakness, it appears to undergo muscle remodelling resulting in increased fatigue resistance. Consequently, preterm infants exposed to an inflammatory environment *in utero* are at increased risk of respiratory muscle weakness that may impede adequate ventilation and contribute to the development of postnatal respiratory failure.