

# Antenatal Corticosteroids Increase Fetal, But Not Postnatal, Pulmonary Blood Flow in Sheep

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**ABSTRACT:** The lungs of very preterm infants have immature airways and gas exchange structures and are usually surfactant deficient. Antenatal corticosteroids are commonly used to enhance fetal lung maturation in preterm infants, but little is known of their effects on pulmonary blood flow (PBF) before and immediately after birth. Our aim was to determine the effects of antenatal betamethasone on PBF before birth and during the postnatal transition in very preterm lambs. Antenatal betamethasone treatment significantly increased mean fetal PBF from  $20.2 \pm 5.1$  to  $84.3 \pm 18.3$  mL/min at 30 h after administration; the PBF waveform was also significantly altered. Mean diastolic PBF increased from  $-38.5 \pm 4.9$  pretreatment to  $-10.2 \pm 11.0$  mL/min at  $\sim 36$  h after the initial betamethasone dose (negative values indicate retrograde flow away from the lungs). Within 10 min after delivery, PBF was similar in control and betamethasone-treated lambs. These data demonstrate that antenatal betamethasone significantly increases fetal PBF and alters the PBF waveform but has little effect on postnatal PBF. (*Pediatr Res* 66: 283–288, 2009)

Corticosteroids are commonly administered to women at risk of preterm labor to reduce the incidence and severity of respiratory distress syndrome (RDS) in very preterm infants (1,2). The effects of antenatal corticosteroids on the immature lung are well established and include structural maturation of the lung, increased surfactant production, and enhanced lung liquid clearance (2–4). However, little is known of the effect that corticosteroids have on fetal pulmonary hemodynamics.

Before birth, pulmonary blood flow (PBF) is low because pulmonary vascular resistance (PVR) is high. As a result, most blood exiting the right ventricle ( $\sim 90\%$ ) bypasses the lungs and flows directly into the descending aorta, *via* the ductus arteriosus (DA) (5). The high PVR and presence of the DA confer a unique shape to the pulmonary artery blood flow waveform, with forward flow only occurring briefly during systole (6,7). During much of diastole, the high PVR promotes “backward” or retrograde flow (away from lungs) with blood exiting the pulmonary circulation *via* the DA (6,7). As a result,

diastolic flows in the left pulmonary artery are a sensitive indicator of changes in PVR (8,9). At birth, the large decrease in PVR greatly increases PBF and markedly changes the contour of the PBF waveform (5–7,10).

In contrast to the mature neonate, little is known about the adaptation of the pulmonary circulation at birth in very preterm infants ( $<28$ -wk gestation). Indeed, it is likely to differ from mature neonates as the immature lung has a poorly developed pulmonary vascular bed (11). As a large cross-sectional surface area is required to provide a low resistance pathway for PBF, if the vessels conferring this attribute have not developed, substantial reductions in PVR cannot be achieved after birth. Very preterm infants commonly suffer from persistent pulmonary hypertension, indicating that PVR remains high in these infants (12). However, as most of these infants are likely to have had antenatal corticosteroids, which can affect blood flow in a variety of vascular beds (13–15), it is possible that corticosteroids influence PBF and the transition of the pulmonary circulation after birth. Our aim was to examine the effects of antenatal betamethasone on PBF and PBF waveform characteristics in the fetus, during the pulmonary transition at birth and for the first 10 min after birth in very preterm lambs.

## METHODS

**Experimental protocol.** All experimental procedures on animals were approved by the Monash University Animal Ethics Committee. Aseptic surgery was conducted on 12 pregnant ewes (Border-Leicester  $\times$  Merino) at  $120 \pm 2$  d of gestational age (GA; term is  $\sim 147$  d). Surgery was performed prenatally so that the effects of corticosteroids on PBF could be examined before, during, and after birth. Catheters were inserted into a fetal carotid artery, jugular vein, amniotic sac, and left pulmonary artery, and a 4-mm ultrasonic flow probe (Transonic Systems; Ithaca, NY) was placed around the left pulmonary artery as described previously (6). Fetal well being was monitored daily by measuring fetal arterial  $PO_2$  ( $PaO_2$ ),  $PCO_2$  ( $PaCO_2$ ), pH, and percent oxygen saturation of Hb ( $SaO_2$ ) (ABL30, Radiometer, Denmark). Blood flow in the pulmonary artery was recorded digitally using a computerized data acquisition system (Powerlab; ADInstruments, Castle Hill, Australia).

At  $125 \pm 2$  d GA, betamethasone (12 mg; Celestone Chronodose; Schering-Plough Pty Limited, Australia) was administered (i.m.) to the ewe ( $n = 6$ ) at 36 h and 24 h before the planned caesarean delivery. The dose and timing of betamethasone administration is similar to the regimen given to women at

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**Abbreviations:**  $FiO_2$ , Fraction of inspired oxygen; **PBF**, Pulmonary blood flow; **PVR**, Pulmonary vascular resistance; **SaO<sub>2</sub>**, Percent oxygen saturation

risk of preterm delivery and induces lung maturation in fetal sheep (16,17). Mean fetal PBF and fetal carotid and pulmonary arterial pressures were recorded for 6 h before betamethasone treatment and the following 36 h before delivery. Ewes did not receive betamethasone in control fetuses ( $n = 6$ ).

At  $127 \pm 2$  d GA, ewes and fetuses were anesthetized, and the fetal head and neck exposed *via* caesarean section. A cuffed endotracheal tube (3.5 mm) was inserted and lung liquid drained before the lambs were delivered, dried, and weighed. Lambs were kept warm ( $39^\circ\text{C}$ ) and ventilated (Babylog 8000+; Dräger, Lübeck, Germany) with a tidal volume ( $V_T$ ) of 5 mL/kg, with a positive end-expiratory pressure (PEEP) of 4 cm  $\text{H}_2\text{O}$ , and at 60 inflations/min. The respiratory rate and fraction of inspired oxygen ( $\text{FiO}_2$ ) were altered to maintain arterial pH (7.30–7.45),  $\text{PaCO}_2$  (35–60 mm Hg), and  $\text{SaO}_2$  (90–95%). Lambs received 5% dextrose (*i.v.*), were sedated (pentobarbital, *i.v.*), and ventilated for the next 2 h (only the first 10 min is reported).

**Analytical methods.** The PBF data were divided into seven 6-h recording blocks, which included the 6-h period before and the 36-h period after betamethasone administration. Within each 6-h recording block, the 5-min periods spanning every 30-min time point (taking time of betamethasone injection as time zero) were analyzed, and the data were averaged over the 6-h block. Changes in the PBF waveform were measured by selecting representative waveforms throughout 10 consecutive cardiac cycles from each fetus before and 36 h after betamethasone administration as well as during the first 10 min after the initiation of ventilation. Waveform parameters examined were mean diastolic PBF and end-diastolic left PBF, as previously described (8). PVR was calculated using the formula  $\text{PVR} = \text{PPA} - \text{PLA}/\text{QP}$ , where PPA is pulmonary arterial pressure, PLA is left arterial pressure, and QP is flow through the left pulmonary artery; PLA was assumed to be 3 mm Hg in the fetus and 9 mm Hg in the lamb based on previous studies (6,8,18).

**mRNA analysis.** To determine the effect of antenatal betamethasone on factors that may regulate PBF, two separate groups of ewes were administered either betamethasone ( $n = 5$ ; 12 mg; *i.m.*) or saline ( $n = 5$ ) at 125 d GA and tissues collected 36 h later.

*eNOS* and *VEGF* mRNA levels in lung tissue were measured using a quantitative real-time polymerase chain reaction (qRT-PCR). The primers used for amplification are *eNOS* Forward 5'CTGGTACATGAGCAGCAGAGA3', *eNOS* Reverse 5'CAGGATGTTGTAGCGGTGAG3' and *VEGF* Forward 5'CGAAAGTCTGGAGTGTGTGC3', *VEGF* Reverse 5'TATGTGCTGGCTTTGGTGAG3'. The gene accession number is Acc# AF201926 for *eNOS* and Acc# NM\_001025110 for *VEGF*, and the region amplified is 639–702 bp for *eNOS* and 264–348 bp for *VEGF*. Total RNA was extracted, DNase-treated, and 1  $\mu\text{g}$  was reverse transcribed into cDNA (M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant Kit; Promega, Madison, WI). The qRT-PCR was performed using a Mastercycler ep gradient S realplex real-time PCR system (Eppendorf, Germany) using 1  $\mu\text{L}$  cDNA template (500 ng/ $\mu\text{L}$  for *eNOS* and for 200 ng/ $\mu\text{L}$  *VEGF*), 1  $\mu\text{L}$  of each forward and reverse primer (10  $\mu\text{M}$ ), 10  $\mu\text{L}$  SYBR green (Platinum SYBR Green qPCR SuperMix-UDG; Invitrogen Life Technologies, Carlsbad, CA), and 7  $\mu\text{L}$  of nuclease-free water. The thermal profile used to amplify the PCR products included an initial 2-min incubation at  $95^\circ\text{C}$ , followed by 35–40 cycles of denaturation at  $95^\circ\text{C}$  for 3 s, annealing at  $60^\circ\text{C}$  for 20 s, and elongation at  $72^\circ\text{C}$  for 20 s. The fluorescence readings were recorded after each  $72^\circ\text{C}$  step. Dissociation curves were performed after each PCR run to ensure that a single PCR product had been amplified per primer set. Each sample was measured in triplicate, and a control sample, containing no template, was included in each run. A threshold value ( $C_T$  value) for each sample was determined. Minor differences in the amount of cDNA template added to each reaction were adjusted by subtracting the  $C_T$  value for 18S from the  $C_T$  value for each sample and gene of interest ( $[\Delta\Delta]C_T$ ). mRNA levels were normalized to 18S rRNA levels and expressed as a fold change relative to the mean mRNA levels in nonventilated control fetuses.

**Statistical analysis.** A one-way repeated measures ANOVA was used to determine the effect of betamethasone on mean PBF before and over the 36-h period after betamethasone administration (Sigmastat; SPSS, Chicago, IL). A paired *t* test was used to analyze mean diastolic and end-diastolic PBF before and after betamethasone administration and *eNOS* and *VEGF* mRNA levels. A *t* test or a Mann-Whitney ranks test (when the data were not normally distributed) was used to analyze hemodynamic parameters (mean arterial pressure, pulmonary artery pressure, PVR, and heart rate) and blood gas values between control and betamethasone-treated fetuses directly before delivery.

Multiple two-way repeated measures ANOVAs were used to determine the effect of betamethasone on mean diastolic, end-diastolic, and mean PBF during the initiation of ventilation and the effect of birth on hemodynamic parameters. Fisher least significant difference (LSD) *post hoc* tests were used to detect differences between the treatment groups and time points. When data were not normally distributed, a Friedman Repeated measures ANOVA on ranks was used. A *t* test or a Mann-Whitney ranks test was used to analyze

blood gas values, mean airway pressure, and peak inspiratory pressure 5 min after the initiation of ventilation. Data in the text are presented as the mean  $\pm$  SEM. The level of statistical significance was  $p < 0.05$  for all statistical analyses.

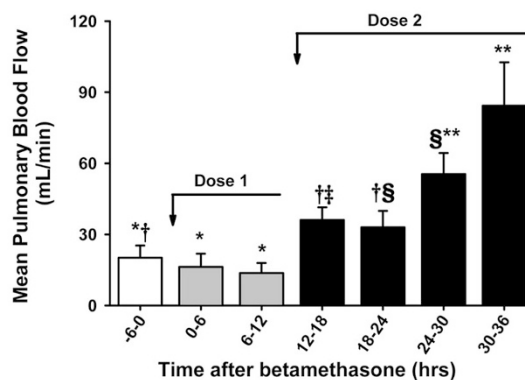
## RESULTS

**Prenatal effects of corticosteroids.** Fetal arterial blood gas and pH values were similar to control fetuses and were within normal physiological ranges before and after antenatal betamethasone administration. Control fetuses: pH,  $7.38 \pm 0.01$ ;  $\text{PaCO}_2$ ,  $43.3 \pm 1.9$  mm Hg;  $\text{PaO}_2$ ,  $18.5 \pm 2.3$  mm Hg;  $\text{SaO}_2$ ,  $61.1 \pm 6.2\%$ ; betamethasone-treated fetuses: pH,  $7.38 \pm 0.2$ ;  $\text{PaCO}_2$ ,  $40.8 \pm 3.7$  mm Hg;  $\text{PaO}_2$ ,  $19.9 \pm 2.8$  mm Hg;  $\text{SaO}_2$ ,  $68.1 \pm 6.3\%$ .

**PBF.** Mean PBF significantly increased ( $p < 0.001$ ; Fig. 1) ~2.5-fold after the first dose of betamethasone, from  $20.2 \pm 5.1$  mL/min before betamethasone to  $55.5 \pm 8.9$  mL/min at 24 h (12 h after the second dose of betamethasone). Mean PBF increased further to  $84.3 \pm 18.3$  mL/min at 30 h, resulting in a 4-fold increase in mean flow, compared with pretreatment values (Fig. 1). These changes in fetal PBF were associated with a significant decrease in PVR between control and betamethasone-treated fetuses (Table 1). There were no differences in the mean carotid arterial and pulmonary arterial pressure or fetal heart rate.

**Diastolic PBF.** Mean diastolic flow in the fetal pulmonary artery significantly increased from  $-39 \pm 5$  to  $-10 \pm 11$  mL/min ( $p = 0.04$ ; Fig. 2A) at ~36 h after the initial dose of betamethasone (just before delivery); a negative value indicates retrograde flow away from the lung. Similarly, end-diastolic PBF was significantly increased from  $-48 \pm 6$  to  $-34 \pm 8$  mL/min at ~36 h after betamethasone treatment,  $p = 0.002$  (Fig. 2B).

**PBF waveform.** The effects of antenatal betamethasone on the contour of the left PBF waveform are displayed in Figure 3. In both control fetuses and fetuses before betamethasone treatment, flow in the pulmonary artery was only positive (flow toward the lung) briefly during systole and was primarily retrograde (indicated by negative PBF values) throughout most of diastole. Betamethasone treatment clearly altered the PBF waveform, increasing peak flow during systole as well as mean flow during diastole (Fig. 3).

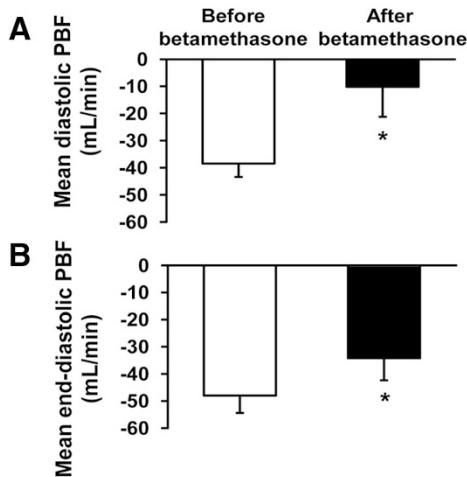


**Figure 1.** Mean fetal PBF (mL/min) before (□) and after (■) antenatal betamethasone treatments shown in 6 h time periods. Values that do not share a common symbol are significantly different from one another ( $p < 0.05$ ).

**Table 1.** Effect of betamethasone on hemodynamic parameters in fetal sheep

Fetal group	Mean arterial pressure (mm Hg)	Pulmonary artery pressure (mm Hg)	Pulmonary vascular resistance (mm Hg · min <sup>-1</sup> · mL <sup>-1</sup> )	Pulmonary blood flow (mL/min)	Heart rate
Control (n = 6)	39.8 ± 1.6	40.7 ± 1.6	3.2 ± 0.8	18.7 ± 10.4	157 ± 8
Betamethasone (n = 5-6)	40.7 ± 1.3	42.9 ± 1.2	0.8 ± 0.2*	88.9 ± 25.9*	187 ± 11

Parameters were averaged over the 6 h directly before caesarean delivery; maternal betamethasone (12 mg i.m.) was given 36 and 24 h before delivery. \*Significant difference compared with control, *p* < 0.03.

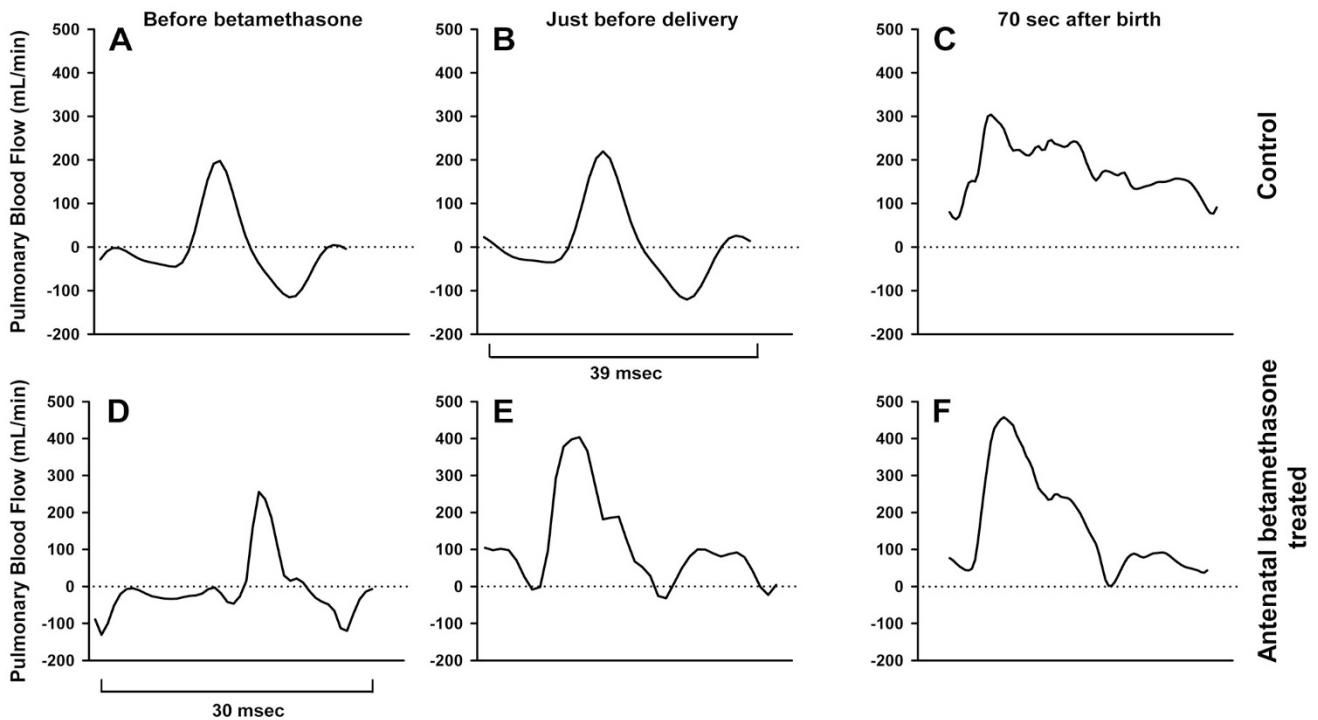


**Figure 2.** Mean fetal diastolic PBF (A) and end-diastolic PBF (B) before (□) and ~36 h after (■) antenatal betamethasone treatments. \**p* < 0.05 before and after betamethasone treatment. Data were averaged over the 6-h time period before betamethasone treatment and the 6-h time period before caesarean delivery.

**Effect of corticosteroids on birth-related changes in PBF.**

Arterial blood gas and pH values were similar in betamethasone-treated lambs compared with control lambs at 5 min after the onset of ventilation. Control lambs: pH, 7.23 ± 0.02; PaCO<sub>2</sub>, 54.0 ± 3.4 mm Hg; PaO<sub>2</sub>, 90.4 ± 25.9 mm Hg; SaO<sub>2</sub>, 93.9 ± 2.8%; FiO<sub>2</sub>, 0.95 ± 0.05; AaDO<sub>2</sub>, 525 ± 57 mm Hg; betamethasone-treated lambs: pH, 7.26 ± 0.06; PaCO<sub>2</sub>, 47.5 ± 7.8 mm Hg; PaO<sub>2</sub>, 35.3 ± 7.1 mm Hg; SaO<sub>2</sub>, 81.1 ± 7.2%; FiO<sub>2</sub>, 0.98 ± 0.003; AaDO<sub>2</sub>, 615 ± 9 mm Hg. Mean airway pressures (13.8 ± 0.4 cm H<sub>2</sub>O versus 13.3 ± 2.0 cmH<sub>2</sub>O) and peak inspiratory pressures (39.8 ± 1.7 cmH<sub>2</sub>O versus 42.0 ± 5.6 cm H<sub>2</sub>O) were similar 5 min after the onset of ventilation in both control and betamethasone-treated lambs.

At birth and before the onset of ventilation, mean flow in the left pulmonary artery was significantly (*p* = 0.003) lower in control lambs (59.0 ± 11.4 mL/min) compared with betamethasone-treated lambs (164.6 ± 37.3 mL/min). Between 10 s and 10 min after the initiation of ventilation, mean PBF in control lambs increased ~3.5-fold (Fig. 4A). In contrast, PBF in betamethasone-treated lambs only increased 1.5-fold over the same time period and, as a result, mean PBF was similar in both groups after 10 min of ventilation (Fig. 4A).



**Figure 3.** Representative waveforms from the left pulmonary artery obtained throughout one cardiac cycle from a control (upper panel: A, B, and C) and antenatal betamethasone-treated animal (lower panel: D, E, and F). Waveforms shown are before betamethasone (A and D), just before delivery (B and E) and 70 s after the initiation of ventilation (C and F).



These changes in PBF were associated with a significant decrease in PVR in both groups of lambs at 10 min, compared with 10 and 70 s after birth; although PVR values were initially much higher in control lambs (Table 2).

There were no differences in mean arterial and pulmonary artery pressures or heart rates between control and betamethasone-treated lambs in the first 10 min after birth (Table 2).

The PBF waveform was markedly altered by the onset of ventilation in both control and betamethasone-treated lambs. At 10 s after initiating ventilation, mean diastolic flows in the pulmonary artery were significantly ( $p = 0.04$ ) lower in control ( $32.1 \pm 19.2$  mL/min) than in betamethasone-treated ( $107.9 \pm 25.2$  mL/min) lambs (Fig. 4B). However, no further differences between the groups were detected after this time. Similarly, end-diastolic PBF was significantly ( $p = 0.02$ ) lower in control ( $-17.3 \pm 18.0$  mL/min) than in betametha-

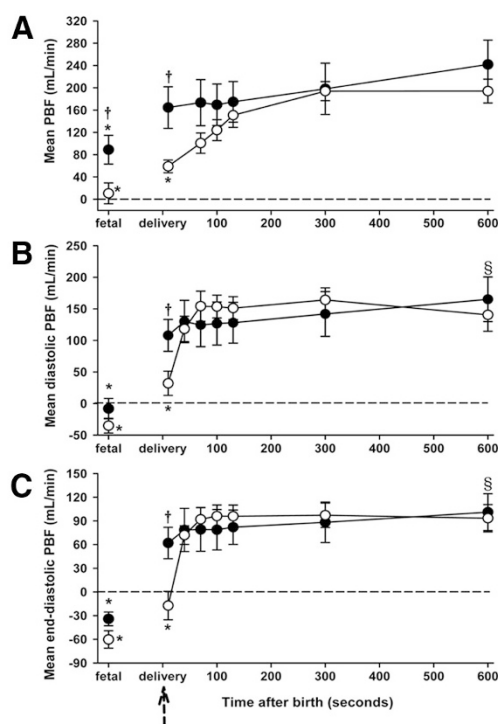
sone-treated ( $62.0 \pm 19.9$  mL/min) lambs at 10 s after the onset of ventilation (Fig. 4C). However, no further differences were detected at subsequent time-points after the onset of ventilation. Indeed, by 70 s after the initiation of ventilation, mean diastolic PBF had increased to  $154.2 \pm 23.7$  mL/min in control fetuses, which was not significantly different from mean diastolic PBF in betamethasone-treated fetuses ( $124.4 \pm 34.3$  mL/min) (Fig. 4B).

**Changes in eNOS and VEGF expression.** The mRNA levels for eNOS and VEGF in fetal lung tissue were not altered by betamethasone treatment. Relative mRNA levels for both eNOS ( $1.00 \pm 0.14$  versus  $1.00 \pm 0.10$ ) and VEGF ( $1.00 \pm 0.08$  versus  $0.96 \pm 0.15$ ) were similar in control and betamethasone-treated fetuses.

## DISCUSSION

Our findings demonstrate that antenatal betamethasone significantly increases PBF in very immature fetal sheep, due to a decrease in PVR, without increasing either eNOS or VEGF expression. As a result, the prenatal PBF waveform was markedly altered, leading to a significant increase in both mean and end-diastolic PBF. However, despite the large increase in prenatal PBF, antenatal corticosteroids seemed to reduce the magnitude of the increase in PBF after birth. Indeed, PBF was similar in control and betamethasone-treated lambs within 2 min of the onset of pulmonary ventilation. As a result, mean PBF increased only 1.5-fold in betamethasone-treated lambs, compared with a 4-fold increase in control lambs, during the prenatal to postnatal transition. These data indicate that antenatal corticosteroids either activate (*in utero*) similar mechanisms as those responsible for the birth-related changes in PBF or activate different mechanisms, which counteract the birth-related mechanisms.

Corticosteroids affect blood flow in a variety of organs, including the placenta (14), kidney (13), and cerebral circulation (15). However, the induced affect and mechanism of action varies considerably depending upon the vascular bed. Corticosteroids vasodilate the umbilical circulation both *in vivo* (14,19) and *in vitro* (20,21) in humans, possibly *via* an endothelium-independent pathway (20). Corticosteroids also reduce vascular resistance and increase blood flow in the adult kidney, but these effects are thought to be mediated *via* endothelial-dependent pathways involving prostacyclin and NO (13). On the other hand, corticosteroids can increase fetal systemic blood pressure by increasing peripheral vascular



**Figure 4.** Effect of ventilation at birth on mean PBF (PBF, A), mean diastolic (B), and end-diastolic PBF (C) in control (○) and antenatal betamethasone-treated (●) lambs. \* $p < 0.05$  for values in all other time points in the control and/or betamethasone group. † $p < 0.05$  between the control and betamethasone-treated fetuses (A) and lambs at the 10-s time point (A, B, and C). § $p < 0.05$  between 10 and 600 s in betamethasone-treated lambs (B and C).

**Table 2.** Effect of birth on hemodynamic parameters in control and betamethasone-treated newborn lambs at 10, 70, and 600 s after the initiation of ventilation

Treatment group	Time after birth (s)	Mean arterial pressure (mm Hg)	Pulmonary artery pressure (mm Hg)	Pulmonary vascular resistance (mm Hg · min <sup>-1</sup> · mL <sup>-1</sup> )	Heart rate
Control (n = 6)	10	44.2 ± 5.0	42.8 ± 3.9	0.86 ± 0.22	157 ± 16
	70	47.4 ± 3.7	42.9 ± 4.1	0.62 ± 0.18	160 ± 14
	600	33.8 ± 4.7	36.4 ± 4.1	0.17 ± 0.02*	159 ± 14
Betamethasone (n = 5–6)	10	44.6 ± 4.9	35.6 ± 6.2	0.24 ± 0.03	178 ± 17
	70	42.7 ± 4.9	33.2 ± 6.0	0.27 ± 0.05	154 ± 11
	600	42.0 ± 2.9	34.7 ± 6.6	0.14 ± 0.04*	166 ± 14

Parameters were averaged over the 10 consecutive PBF waveforms at the times indicated.

\* Significant difference compared with 10 and 70 s within that group,  $p < 0.01$ .

resistance (22,23) and decrease cerebral blood flow by increasing cerebral vascular resistance (15).

Corticosteroids have also been shown to influence the fetal pulmonary circulation by enhancing its adaptation at birth. In particular, corticosteroids can increase pulmonary vascular reactivity in response to vasodilatory stimuli, including catecholamines (24,25) prostaglandins, and NO (24,26). However, previous studies found that antenatal corticosteroids have no effect on basal PBF in fetal sheep (25,27) and were considered to enhance pulmonary vasodilation induced by alveolar ventilation at birth; this effect was independent of oxygen-mediated changes. These data contrast with our findings of a marked increase in fetal PBF in response to antenatal corticosteroids, with little or no beneficial effect on the transition of the pulmonary circulation at birth. The difference between our findings and those of previous studies (25,27) is likely because of the maturity of the fetus when exposed to corticosteroids. In previous studies, fetuses were treated at 135–138 d of gestation (25,27), whereas in our study, fetuses were treated at ~125 d; term is ~147 d.

In sheep, the alveolar stage of lung development begins at ~120 d of gestation and the lungs develop millions of alveoli surrounded by a dense alveolar capillary network by term (28). The resulting increase in cross-sectional surface area of the capillary vascular bed explains the gradual reduction in PVR over this time (7). Considering the marked development of the pulmonary vascular bed, it is not surprising that the responses to corticosteroids are different at 125 and 135 d of gestation. However, we consider that our results are more applicable to preterm human infants exposed to antenatal corticosteroids as the infants that develop RDS are mostly born during the late canalicular, sacular, or early alveolar stages of lung development. Indeed, our results are consistent with the finding that antenatal corticosteroids induce a reduction in fetal pulmonary artery resistance in human fetuses as measured using Doppler echocardiography (29).

The mechanisms by which antenatal corticosteroids increase PBF before birth are unknown, but must result in a decrease in PVR, as fetal pulmonary arterial pressure and heart rate were not changed. This suggestion is consistent with our finding that antenatal corticosteroid treatment caused a significant increase in mean diastolic and end-diastolic fetal PBF. We have previously reported that changes in mean diastolic and end-diastolic flow in the left pulmonary artery is a sensitive indicator of down stream resistance in the pulmonary vascular bed (8,9). Flow during diastole is largely determined by resistance (and compliance of the arterial walls) and, therefore, more reliably predicts PVR than a mean of the flow over the cardiac cycle. Thus, it is highly likely that antenatal corticosteroids promote vasodilation of the pulmonary vascular bed in the immature lung, possibly by increasing the responsiveness to vasodilators, such as NO, or by increasing the production/release of these vasodilators (30). It is interesting to note that pulmonary arterial pressure tended to be lower in corticosteroid-treated lambs after birth (Table 2) but was markedly lower in relation to systemic arterial pressure (on average 9 mm Hg *versus* 1.2 mm Hg). This likely reflected the lower PVR in corticosteroid-treated lambs and the resulting

pressure gradient must have caused major left-to-right shunting of blood through the DA from the systemic circulation.

Although we could not detect a change in VEGF or eNOS expression in response to corticosteroids, it is possible that increases in expression occurred much earlier than the single time point examined. It may have been more appropriate to measure the activity of nitric-oxide-cGMP and/or prostacyclin-cAMP pathways directly. However, it is unlikely that corticosteroids induced accelerated development of the pulmonary vascular bed, leading to an increase in cross-sectional surface area, as this would have been expected to produce a greater increase in PBF after birth, compared with controls. This suggestion is consistent with findings that antenatal betamethasone did not increase medial wall thickness or volume density of the lumen of small pulmonary arteries in neonatal lambs with hypoplastic lungs (31) and further supporting our suggestion that antenatal betamethasone does not cause proliferation or increased development of the pulmonary vascular bed.

At birth, the mechanisms leading to the large decrease in PVR and increase in PBF are thought to be multifactorial (5,7,10). These include the release of potent vasodilators such as NO and prostacyclin (30), increased oxygen tension, and the mechanical effect of ventilation (10,18). The latter likely results from changes in the distribution of force within perialveolar tissue caused by lung aeration, the formation of surface tension, and the resulting increase in lung recoil (8,32,33). Our finding, that antenatal corticosteroids increase prenatal PBF but have minimal affect on the increase in PBF at birth, indicates that antenatal corticosteroids may activate similar mechanisms as those normally activated at birth in the immature lung. This may explain why PBF was found to increase only 1.5-fold, in corticosteroid-treated fetuses compared with a 4-fold increase in control fetuses. However, a previous study found that antenatal corticosteroids only had a positive affect on postnatal PBF and PVR when the fraction of inspired oxygen ( $FiO_2$ ) was  $<1.0$  (27). As all fetuses were initially ventilated with 100% oxygen, which was then reduced as required to maintain oxygenation, it is possible that a significant effect of antenatal corticosteroids on postnatal PBF will have been observed if the lambs were initially ventilated in air. The relationship between  $FiO_2$  and the dynamics of PBF changes at birth have not been well explored, particularly in the presence and absence of antenatal corticosteroids.

As antenatal glucocorticoids administration has adverse effects on alveolarisation in fetal sheep (34,35) and rats (36), it is also possible that betamethasone reduced alveolar capillary development in our study, thereby reducing the capacity of the pulmonary circulation to transition at birth. Infants born very preterm are at high risk of developing bronchopulmonary dysplasia (BPD), which is characterized by disrupted alveolar development and impaired vascular growth (11). As many infants suffering from BPD are exposed to antenatal glucocorticoids, it is possible that alveolar capillary development is reduced in these infants thereby limiting the increase in PBF that can occur at birth. Thus, because betamethasone-treated lambs only demonstrated a 1.5-fold increase in PBF at birth,

this may be due to a reduced cross-sectional area of the pulmonary vascular bed.

In conclusion, we have shown that antenatal corticosteroids significantly increase fetal PBF in the immature lung, due to a decrease in PVR, but reduce the transition of the pulmonary circulation at birth, resulting in similar PBFs within minutes of birth. The reduction in PVR is most likely due to an increase in responsiveness to vasodilatory stimuli.

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